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CALCIFICATION AND OSSIFICATION

MOBILIZATION OF BONE SALT BY PARATHYROID EXTRACT

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In the course of studies on the mode of action of parathyroid extract on bone we have observed bone salt in transit from the disintegrating trabeculae of spongy bone to the venules of the marrow. These observations were made possible by a routine previously described for serial sectioning of undecalcified bone and its staining by an adaptation of the von Kóssa method.[1] Histologically demonstrable mobilization of bone salt following injection of single doses of parathyroid extract was observed in four experiments on puppies. It was produced in young rats by the same agent but only under the particular conditions to be described. It was also observed in rats after administration of calciferol and of dihydrotachysterol.[2]

The same histologic routine has permitted study of the time relationships in the resorption of the organic matrix and inorganic salts of bone and of the reported phagocytic function of osteoclasts in the transfer of minerals from bone to blood. We have found, as did Kölliker,[3] that bone salt and bone matrix are resorbed simultaneously, and have found no evidence of a phagocytic function of osteoclasts. We have, however, seen that other cells of the bone marrow, chiefly macrophages, may take up bone salt in transit to the blood vessels.

The present paper reports only the experiments in which mobilization of bone salt was demonstrated histologically and the findings as to the mobilization of bone salt and as to the relation of osteoclasts and other

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1. McLean, F. C., and Bloom, W.: Anat. Rec. **78**:333, 1940.

2. Morris, W. D., and McLean, F. C.: To be published.

3. Kölliker, A.: Die normale Resorption des Knochengewebes und ihre Bedeutung für die Entstehung des typischen Knochenformen, Leipzig, F. C. W. Vogel, 1873.

cells to this phenomenon. More detailed chemical and histologic studies of the mode of action of parathyroid extract on bone will be published separately.

EXPERIMENTS ON PUPPIES

The experimental procedure and findings in 18 mongrel puppies are summarized in table 1.

In each experiment the members of one litter were given by subcutaneous injection single doses of parathyroid extract and were killed at intervals thereafter; one member of each litter, as a control, was not given the extract. Blood for analysis was taken by cardiac puncture just before each animal was put to death.

Bones were fixed in 4 per cent neutral solution of formaldehyde, dehydrated in neutral alcohol, embedded in nitrocellulose and cut, without decalcification, in thin

TABLE 1.—*Mobilization of Bone Salt in Puppies After Single Doses of Parathyroid Extract*

Puppy *	Age, Days	Weight, Kg.	Dose of Parathyroid Extract, Units per Kg.	Time, Hr.	Serum		Mobilization	
					Ca, mM per Liter	P, mM per Liter	Extracellular	Intracellular
8101	35	0.82	(Control)	..	2.91	2.36	0	0
8102	35	0.96	200	8	3.88	....	0	0
8103	35	0.97	200	12	4.21	2.36	++	0
8104	35	0.78	200	24	4.06	....	++	++
8105 †	35	0.85	200	48	....	....	0	+
9801	46	1.00	(Control)	..	2.85	3.91	0	0
9802	46	0.74	250	2	2.95	3.16	0	0
9803	46	0.84	250	4	3.58	3.19	0	0
9804	46	1.20	250	6	4.02	3.06	0	0
9805	46	1.29	250	8	4.27	3.38	0	0
9806	46	1.21	250	12	4.64	3.53	0	0
9807	46	0.97	250	24	4.47	3.06	++++	++++
14201	38	2.16	(Control)	..	2.68	3.60	0	0
14202	38	2.23	150	24	4.25	4.00	++++	+
14203 †	38	2.26	150	31	....	....	+	++
14801	34	1.77	(Control)	..	2.60	3.28	0	0
14802	34	1.52	100	24	4.39	3.15	0	0
14803 †	34	1.94	100	48	....	....	+	+

* In the series beginning with puppies 8101, 9801 and 14201 only the costochondral junctions were examined without decalcification. In the series beginning with puppy 14801 the costochondral junctions, radius and tibia were examined.

† This puppy was found dead.

(usually 10 micron) serial sections. They were impregnated with silver nitrate and counterstained with hematoxylin and eosin.[1] Other bones, including the corresponding bones from the other side of the animal, were fixed in Zenker's solution prepared with formaldehyde instead of acetic acid, embedded in nitrocellulose, decalcified in 3 per cent nitric acid and stained with hematoxylin-eosin-azure II and by the Mallory-azan method.[4]

Serum calcium was determined by the method of Kramer and Tisdall[5] as modified by Clark and Collip.[6] Serum phosphate was determined by the method of Fiske and Subbarow.[7]

4. McClung, C. E.: Handbook of Microscopical Technique, ed. 2, New York, Paul B. Hoeber, Inc., 1937.

5. Kramer, B., and Tisdall, F. F.: J. Biol. Chem. **48**:223, 1921.

6. Clark, E. P., and Collip, J. B.: J. Biol. Chem. **63**:461, 1925.

7. Fiske, C. H., and Subbarow, Y.: J. Biol. Chem. **66**:375, 1925.

General Observations.—None of the puppies allowed to live more than twenty-four hours survived for as long as forty-eight hours, indicating that the doses used (100 to 250 units per kilogram of body weight) were severely toxic and would probably have proved lethal in all the animals that received injections. At the end of twenty-four hours the puppies receiving the larger doses were sick and in obvious pain and could not stand. At autopsy at this stage there were subperiosteal hemorrhages in the ribs near the costochondral junctions and separation of the substantia spongiosa from the epiphysial cartilage disks in the long bones. The histologic changes in the bones of all the puppies were those of the early stages of hyperparathyroidism,[8] with extensive resorption of the substantia spongiosa following the larger doses within twenty-four hours.

Mobilization of bone salt was not observed in 5 puppies examined less than twelve hours after injection. It was demonstrated in 1 of 2 animals at twelve hours and in 3 of the 4 animals put to death at twenty-four hours. It was also visualized in the 3 animals dying thirty-one to forty-eight hours after injection.

Histologic Observations on Bone Sections.—(*a*) At Less Than Twelve Hours: Five puppies were examined less than twelve hours after the administration of parathyroid extract. None of these had demonstrable mobilization of bone salt in their bones. In decalcified sections stained with hematoxylin–eosin–azure II, however, there were foci of necrosis in the bone marrow, with phagocytosis of cellular debris by macrophages, as early as four hours. By this time, also, many of the osteoblasts in the substantia spongiosa were spindle shaped.[8] By eight hours, in the 2 animals examined at this time these changes had progressed and there was, in addition, active resorption of bone, especially in the primary substantia spongiosa.

(*b*) At Twelve Hours: Puppy 8103 was killed twelve hours after subcutaneous injection of 200 units of parathyroid extract per kilogram of body weight. In decalcified sections stained with hematoxylin–eosin–azure II there were areas of necrosis in the marrow, with phagocytosis of cellular debris by macrophages. There was extensive resorption of bone, with increased numbers of osteoclasts, especially in the primary substantia spongiosa.

In undecalcified sections of a costochondral junction impregnated with silver nitrate and counterstained with hematoxylin and eosin, the black-stained trabeculae of bone in both the primary and the secondary substantia spongiosa were covered with small black-staining crystals. These crystals were usually in contact with the trabeculae, giving the surfaces a ragged appearance, but in many places they were detached and appeared as discrete bodies. In the latter places they were usually present in a basophil matrix continuous with the bone and apparently representing the last stages of its disintegration. Here the crystals appeared much like those which Bloom and Bloom[9] observed in undecalcified sections of bone treated for five minutes with Müller's fluid (an aqueous solution of 25 per cent potassium dichromate and 1 per cent sodium sulfate); in both cases the crystals had lost their continuity with the densely calcified trabeculae of bone. In some places where osteoclasts were in contact with disintegrating trabeculae of bone, the junction of the osteoclast with the bone was obscured by the crystals, but

8. McLean, F. C., and Bloom, W.: Science **85**:24, 1937.

9. Bloom, W., and Bloom, M. A.: Anat. Rec. **78**:497, 1940.

there was no aggregation of the crystals in the cytoplasm of the osteoclast (fig. 2*A*). In decalcified sections stained with hematoxylin–eosin–azure II, the junction between the osteoclasts and bone remained clearly defined.

In an area of considerable size in the secondary substantia spongiosa the crystals had lost all relation to the trabeculae and were diffusely distributed in the marrow between the bone and the larger venules, which are ordinarily found approximately midway between trabeculae. Here the crystals were seen to be in association with a basophil intercellular substance staining purple with hematoxylin, in part finely granular and in part appearing as a poorly defined network. Although black-staining crystals, associated with this network, impinged on cells of all kinds, they were in no place aggregated within cells.

In decalcified sections from puppy 9806, following a larger dose of parathyroid extract, both the necrosis of the marrow and the resorption of the spongy bone were farther advanced, and there was beginning collapse of the primary substantia spongiosa. In the necrotic marrow there were many macrophages loaded with cellular debris. In undecalcified sections impregnated with silver nitrate, the same ragged appearance of the disintegrating primary substantia spongiosa was seen as in the case of puppy 8103, with here and there areas of hematoxylin-stained disintegrating bone matrix, containing black crystals. There were, however, no crystals in the marrow, and the purple-staining network described in the foregoing paragraph was not demonstrable.

(*c*) At Twenty-Four Hours: At this time the findings varied roughly with the dose of parathyroid extract, but in 3 of 4 animals killed at this time mobilization of bone salt was demonstrable. The findings characteristic of this stage are illustrated from puppy 9807, following injection of 250 units of parathyroid extract per kilogram of body weight.

In decalcified sections of a costochondral junction stained with hematoxylin–eosin–azure II, there was extensive focal necrosis in the bone marrow, with active phagocytosis of cellular debris, advanced resorption of the substantia spongiosa and shaft, and complete collapse of the primary substantia spongiosa. The number of osteoclasts was increased but was not large in proportion to the extensive destruction of bone. No cells recognizable as osteoblasts remained, the trabeculae being covered with spindle-shaped cells. There were extensive hemorrhages under the periosteum. In the area of mechanical injury and collapse in the spongy bone there were abundant deposits of fibrin. In addition, and especially in association with areas of necrosis in the marrow, there were numerous fine radiating networks of fibrin, frequently seen in close association with bone and beneath the layers of cells covering the bone. There were few heterophil leukocytes in the areas of injury and necrosis. Many bone cells were seen in the process of liberation from bone under resorption, with and without the presence of osteoclasts. In similarly stained sections from the distal end of the radius the findings were identical, the entire secondary substantia spongiosa being separated from the remnants of the primary substantia spongiosa.

Figure 1 illustrates a low power view of an undecalcified section through a costochondral junction from the same puppy, impregnated with silver nitrate and counterstained with hematoxylin and eosin. In the areas where the trabeculae have disappeared there is no bone salt free in the marrow. Deeper in the secondary substantia spongiosa there is widespread mobilization of the bone salt, in part diffusely distributed through the marrow and in part intracellular.

Under higher power (fig. 2 *B*), many cells are seen to be packed with bone salt; in the low power view (fig. 1 *g*) they are visible as discrete aggregations of black-stained material. Many of the cells are so full of the mineral that their characteristics are obscured, but when, because of a smaller content of bone salt,

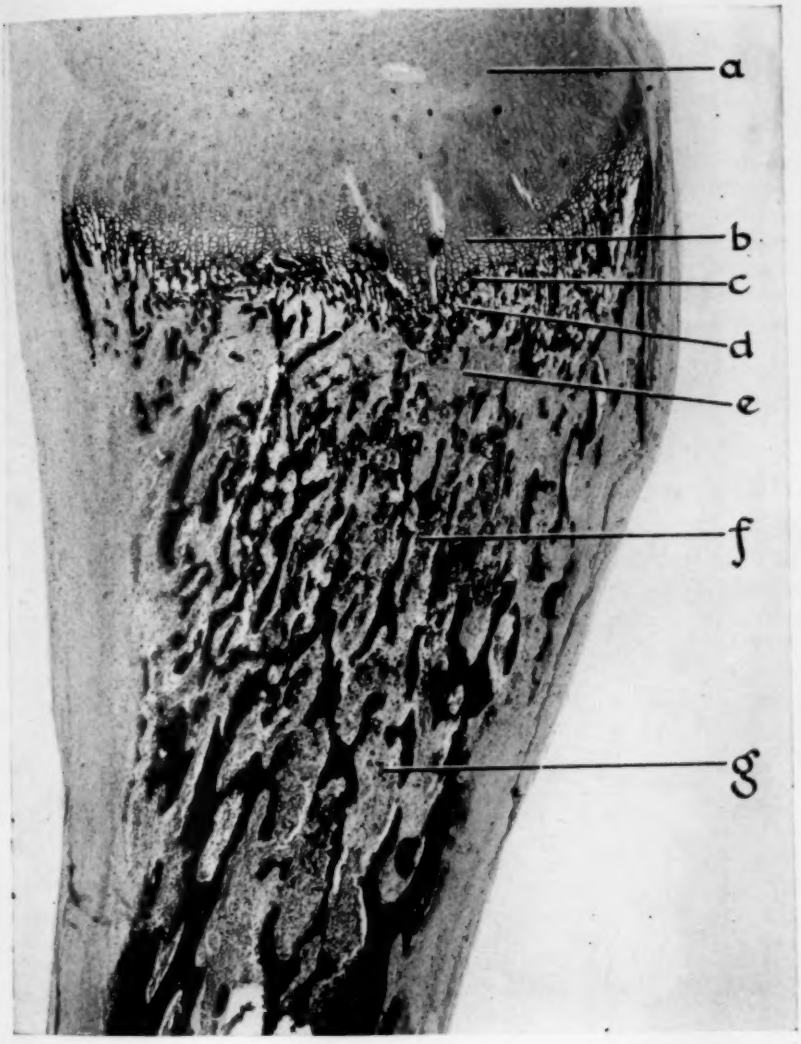

Fig. 1.—An undecalcified section through a costochondral junction of puppy 9807 twenty-four hours after an injection of 250 units of parathyroid extract per kilogram of body weight: (*a*) costal cartilage; (*b*) proliferating cartilage; (*c*) zone of provisional calcification; (*d*) disintegrating substantia spongiosa; (*e*) area of completed resorption, free from bone salt; (*f*) mobilized bone salt diffusely permeating the bone marrow; (*g*) mobilized bone salt aggregated in macrophages. Formaldehyde fixation; 10 microns; silver nitrate, hematoxylin and eosin; photomicrograph, × 25.

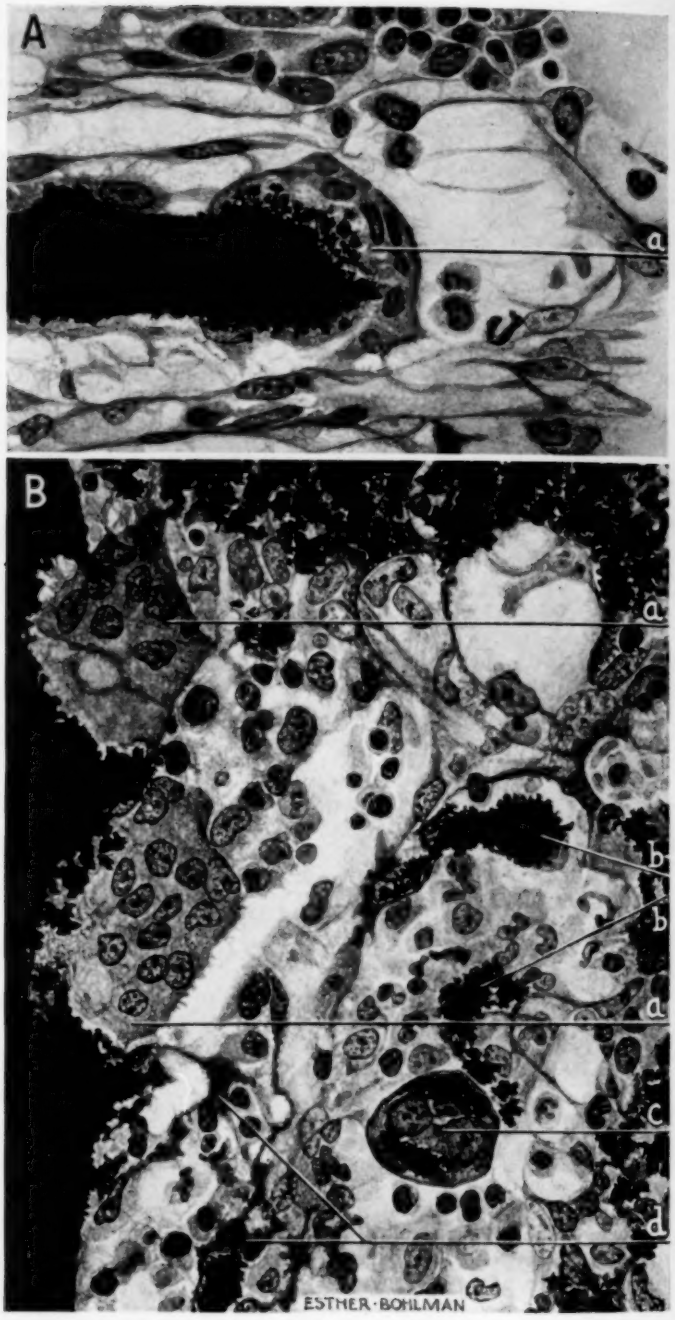

Figure 2

(See legend on opposite page)

they are recognizable, they are usually seen to be macrophages. Many such cells contained both bone salt and cellular debris; many contained debris only. As described at twelve hours (fig. 2 *A*), where osteoclasts were in contact with disintegrating bone the junction between the osteoclast and the bone, although clearly defined in decalcified sections, was often partially obscured by crystals of bone salt separated from the main body of bone, but there was no aggregation of the salt within the osteoclasts (fig. 2 *B*). The salt was occasionally seen within the bodies of other myeloid cells, including megakaryocytes (fig. 2 *B*, *c*).

The black-staining crystals which were not intracellular were associated with a basophil network, staining purple with hematoxylin, more clearly defined than at twelve hours and typical of the puppies as well as of the rats in which bone salt was found diffusely distributed in the marrow. This network was not demonstrable in the bones of the control animals, but very closely resembled that described by Bloom and Bloom [9] (see their fig. 3) in embryonic bone and assumed by them to represent calcified areas from which the bone salt had leached during fixation. This network was clearly distinguishable from the fine networks of fibrin and from the coarser fibrin deposited in areas of mechanical injury, both of which are described in an earlier paragraph and neither of which contained deposits of bone salt. It was found in association with bone under resorption rather than with foci of necrosis in the marrow. Although arranged in strands, it had a granular or amorphous structure, not sufficiently regularly fibrillar and branching for fibrin and distinguishable also from reticular fibers, particularly as seen in the Mallory-azan preparations. It is clear that this network formed a substrate in which calcium salts were deposited; its probable nature and origin will be discussed later in this paper.

In the sections from puppy 14202, killed twenty-four hours after injection of 200 units of parathyroid extract per kilogram of body weight, there was extensive permeation of the marrow with crystals of bone salt, nearly all extracellular (fig. 3 *A*). Here again the crystals were closely associated with a network staining purple with hematoxylin and seen in Mallory-azan preparations to be amorphous or granular rather than fibrillar. There was extensive necrosis of the marrow, as well as complete collapse of the primary substantia spongiosa. In the areas where this collapse had led to mechanical injury there were dense deposits of fibrin.

In sections from puppy 8104 the distribution of the mobilized bone salt was not so widespread as in the preparations just described. That found was in part intracellular and in part associated with a basophil network. Advanced necrosis

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EXPLANATION OF FIGURE 2

A, undecalcified section of a rib of puppy 8103 twelve hours after an injection of 200 units of parathyroid extract per kilogram of body weight: (*a*) osteoclast surrounding the tip of a spicule of bone undergoing disintegration. The junction of the osteoclast with the bone is obscured by crystals of bone salt, but no bone salt is aggregated in the body of the osteoclast. Formaldehyde fixation; 10 microns; silver nitrate, hematoxylin and eosin; camera lucida; × 681.

B, high power view from another section of the rib shown in figure 1: (*a*) osteoclasts, free from aggregated bone salt; (*b*) macrophages, packed with bone salt; (*c*) megakaryocyte, containing bone salt; (*d*) basophil network. Camera lucida, × 681.

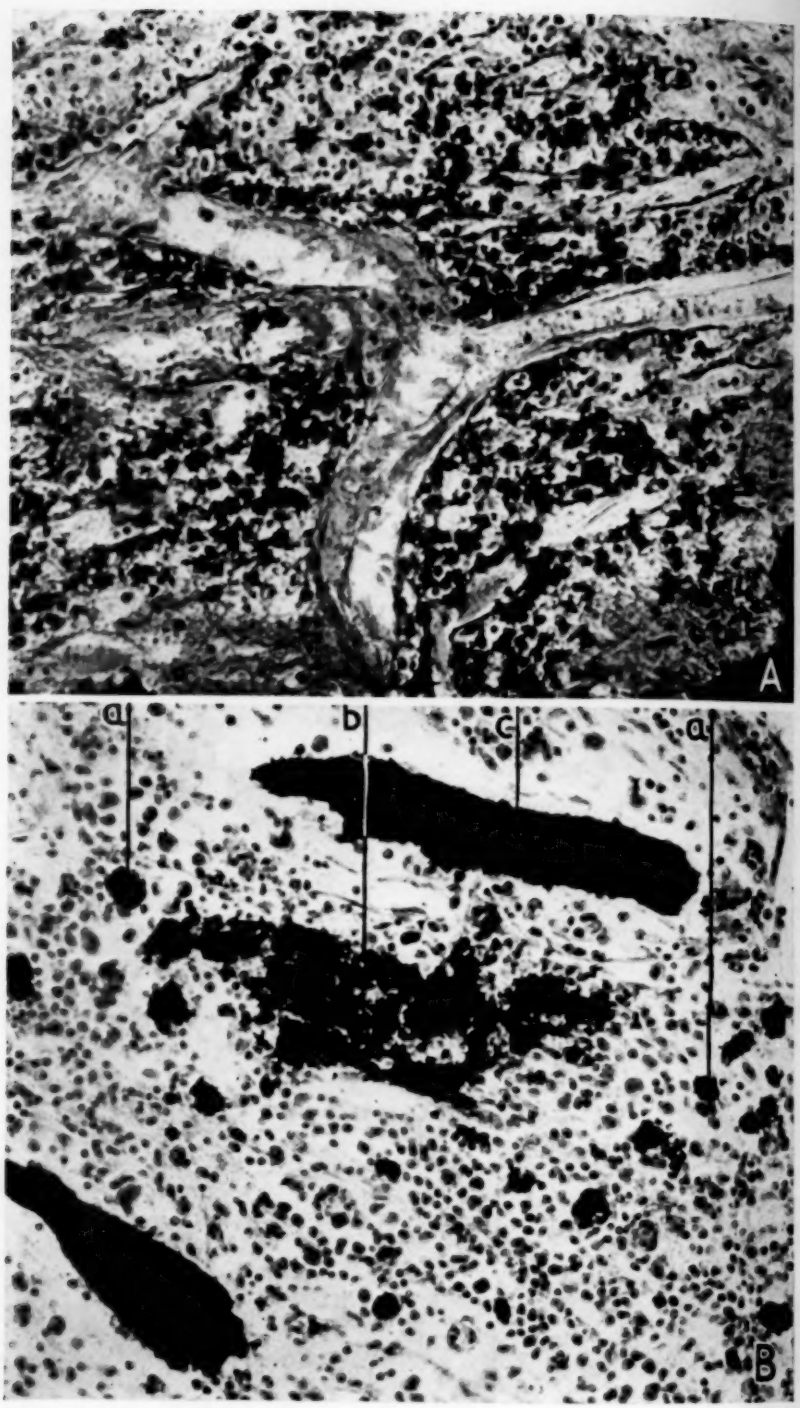

Figure 3

(See legend on opposite page)

of the marrow and resorption of bone were also present. In sections from puppy 14802, killed twenty-four hours after administration of 100 units of parathyroid extract per kilogram of body weight, the smallest dose employed in this series, advanced resorption of bone and collapse of the substantia spongiosa were absent. There was little necrosis in the marrow, and no mobilization of bone salt was demonstrable in the rib, radius or tibia.

(*d*) At Thirty-One to Forty-Eight Hours: No puppy given 100 to 250 units of parathyroid extract per kilogram of body weight survived for forty-eight hours. Puppy 14203 was found dead thirty-one hours after injection of 150 units per kilogram. The sections showed extensive collapse of the primary substantia spongiosa of its bones and widespread necrosis in the marrow, with phagocytosis of cellular debris by macrophages. In a silver preparation of a nondecalcified rib there was mobilized bone salt, in part aggregated within macrophages and in part associated with a network staining purple with hematoxylin (fig. 3 *B*). Some of the macrophages contained both bone salt and cellular debris; many contained cellular debris only.

Puppy 14803 was found dead forty-eight hours after injection of 100 units per kilogram and might have been dead for several hours. In sections of a costochondral junction impregnated with silver nitrate and counterstained with hematoxylin and eosin, there was healing in the area from which the primary substantia spongiosa had been largely resorbed, accompanied by returning osteoblastic activity and the formation of new bone matrix, as yet uncalcified. Deeper in the substantia spongiosa the evidences of injury predominated, with areas of necrosis but without mobilized bone salt. In the distal ends of the radius and tibia there was less repair, with many areas of necrosis and a moderate amount of bone salt, in part phagocytosed and in part associated with a purple-staining network. In the Mallory-azan preparations, collagenous fibers being laid down in the newly forming bone matrix were seen. The network in the marrow stained a pale blue and was arranged in strands, but in contrast with the collagenous fibers did not stain as deeply or display as fibrillar an appearance.

Puppy 8105 was found dead forty-eight hours after injection of 200 units per kilogram, and the body was warm when found. In the sections there were evidences both of necrosis of the marrow and of beginning repair, including new bone formation. Little bone salt was found outside of the trabeculae; that which was present was aggregated in macrophages.

EXPLANATION OF FIGURE 3

A, undecalcified section of a rib of puppy 14202 twenty-four hours after an injection of 150 units of parathyroid extract per kilogram of body weight. Mobilized bone salt may be seen diffusely permeating the bone marrow, with none aggregated in cells. The dark-staining points in the lumen of a blood vessel are nuclei of leukocytes stained with hematoxylin. Formaldehyde fixation; 10 microns; silver nitrate, hematoxylin and eosin; photomicrograph, × 265.

B, undecalcified section of a rib of puppy 14203, which was found dead thirty-one hours after an injection of 150 units of parathyroid extract per kilogram of body weight: (*a*) bone salt aggregated in macrophages; (*b*) mobilized bone salt, in part aggregated in macrophages and in part diffuse; (*c*) bone. Formaldehyde fixation; 10 microns; silver nitrate, hematoxylin and eosin; photomicrograph, × 265.

Other Tissues.—Other organs from the animals of the series beginning with puppies 8101, 14201 and 14801 were fixed in Zenker's formaldehyde solution and sections were stained with hematoxylin–eosin–azure II and by the Mallory-azan method. The thymus, spleen, adrenals, heart, lung, pancreas, intestines, liver and kidney were examined.

The most striking findings were in the thymus and spleen of the animals given parathyroid extract. In both organs there was marked depletion of lymphocytes, with many necrotic and dying lymphocytes present. Focal necrosis in the heart muscle, diffuse myocarditis and acute pancreatitis were seen in the animals found dead. Focal necrosis was present in the midzones of the liver lobules in several animals. There were small amounts of necrosis in the kidneys, with many tubular casts. There is no evidence of calcification in the sections studied. Silver nitrate preparations were not made.

EXPERIMENTS ON RATS

More than 150 rats were treated with parathyroid extract in varying doses and under varying conditions. The histologic procedures were the same as those described for the experiments on puppies (p. 316). In addition, bones were fixed by the Altmann-Gersh freezing-drying method.[9a]

Mobilization of bone salt was demonstrated in 5 rats from three litters. In no case was it seen to follow a single large dose of the extract, although in many instances as much as 1,000 units was administered within a period of four hours. The experiments in which demonstrable mobilization was induced are summarized in table 2.

Rats 6301-6305.—In rats 6304 and 6305 demonstrable mobilization followed intraperitoneal administration of parathyroid extract at the rate of 100 units twice a day for three and four days, respectively. Two other rats from the same litter died after the same dosage for three days and were not examined. It is our experience that the majority of rats at this age die after three to four days of this regimen. The conditions of this experiment differed from many other experiments, in which mobilization of bone salt was not demonstrated, only in that these rats were transferred at the age of 49 days to a low phosphorus diet, the administration of parathyroid extract being begun two days later.

The bones from rats 6302 and 6303 showed increasing transformation of osteoblasts to spindle-shaped cells, without necrosis of the marrow, without increased resorption and without demonstrable mobilization of bone salt. In sagittal sections through the knee joint of rat 6304, which had been given 600 units of parathyroid extract in seventy-two hours, there was extensive and advanced resorption of the substantia spongiosa, leaving large areas adjacent to the epiphysial cartilage disks of the femur and tibia free from bone. The areas formerly occupied by spongy bone were filled in with spindle-shaped cells, among which were large numbers of osteoclasts. Deeper in the substantia spongiosa the trabeculae were in process of active resorption, and there were numerous areas of focal necrosis in the marrow, containing large amounts of cellular debris, in part phagocytosed by macrophages. All of the osteoblasts, prominent in the control animal of the litter, had been replaced by spindle-shaped cells, covering the remaining bone to a depth of several cells. The picture is typical of the height of the resorptive stage of osteitis fibrosa, with extensive production of fibrous tissue.

9a. Gersh, I.: Anat. Rec. **53**:309, 1932.

Figure 4*A* illustrates a low power view of a tangential section through the head of the tibia of this rat, cut without decalcification, impregnated with silver nitrate and counterstained with hematoxylin and eosin. The area in which resorption of substantia spongiosa has been completed is free from bone salt (*c*). At a greater distance from the epiphysial cartilage resorption of the substantia spongiosa is actively in process, and in this area there is widespread deposition of the bone salt in the marrow (*d*). Under higher power this salt is seen to be in the form of crystals in association with a purple-staining network such as that described in the similar material from puppies. Most of the salt is extracellular, but the crystals in the intercellular network frequently impinge on adjacent cells of all kinds. There are also considerable numbers of phagocytic cells, some

TABLE 2.—*Mobilization of Bone Salt in Rats*

Rat *	Age, Days	Weight, Gm.	Dosage of Parathyroid Extract	Total Dose, Units	Time from 1st Dose, Hr.	Mobilization	
						Extracellular	Intracellular
6301	54	112	(Normal control)	0	..	0	0
6302	51	136	100 units twice daily	200	24	0	0
6303	52	155	100 units twice daily	400	48	0	0
6304	53	147	100 units twice daily	600	72	++++	++
6305	54	142	100 units twice daily	800	96	++++	++
9601	27	64	(Normal control)	0	..	0	0
9602	27	43	50 units twice daily	300	72	0	0
9603	27	40	100 units twice daily	200	24	++	++
9604	27	44	100 units twice daily	400	48	++	++
10901	53	106	(Normal control)	0	..	0	0
10902	52	94	100 units twice daily	600	72	0	0
10903	52	75	100 units twice daily	600	72	0	0
10904	53	120	(Dietary control)	0	..	0	0
10905	50	100	100 units twice daily	200	24	0	0
10906	52	80	100 units twice daily	600	72	+	+
10907	52	78	100 units twice daily	700	84	0	0
10908	53	102	100 units twice daily	900	102	0	0

* In each case the distal end of the femur and the proximal end of the tibia were examined without decalcification. Two additional rats from the series beginning with rat 6301 and two from that beginning with rat 9601 died and were not examined. Animals 6302 to 6305 and 10904 to 10908 were transferred from fox chow to the Steenbock-Black low phosphorus rachitogenic diet as modified by Hess, Weinstock, Rivkin and Gross,[10] two days before the administration of parathyroid extract was begun.

loaded with dense aggregations of the silver salt, some containing silver salt and debris, and some containing debris only. There are large numbers of osteoclasts, with here and there a random black-staining crystal seen within the cytoplasm, but there is nowhere any aggregation either of cellular debris or of bone salt within these cells. This preparation is also noteworthy for the association of the bone salt with some of the venules of the marrow, shown in higher power in figure 4*B*. Here long stretches of the endothelium of the venules are obscured by the dense deposit of silver.

The sections from rat 6305, killed at eighty-four hours, showed a later stage of the process just described, with advanced resorption of the substantia spongiosa and shaft. Osteoclasts were numerous in the areas from which bone had been resorbed and in contact with the compact bone of the shaft undergoing resorption. There were numerous areas of necrosis in the marrow. Bone salt, stained with silver, was found in the marrow, both as crystals in association with a basophil

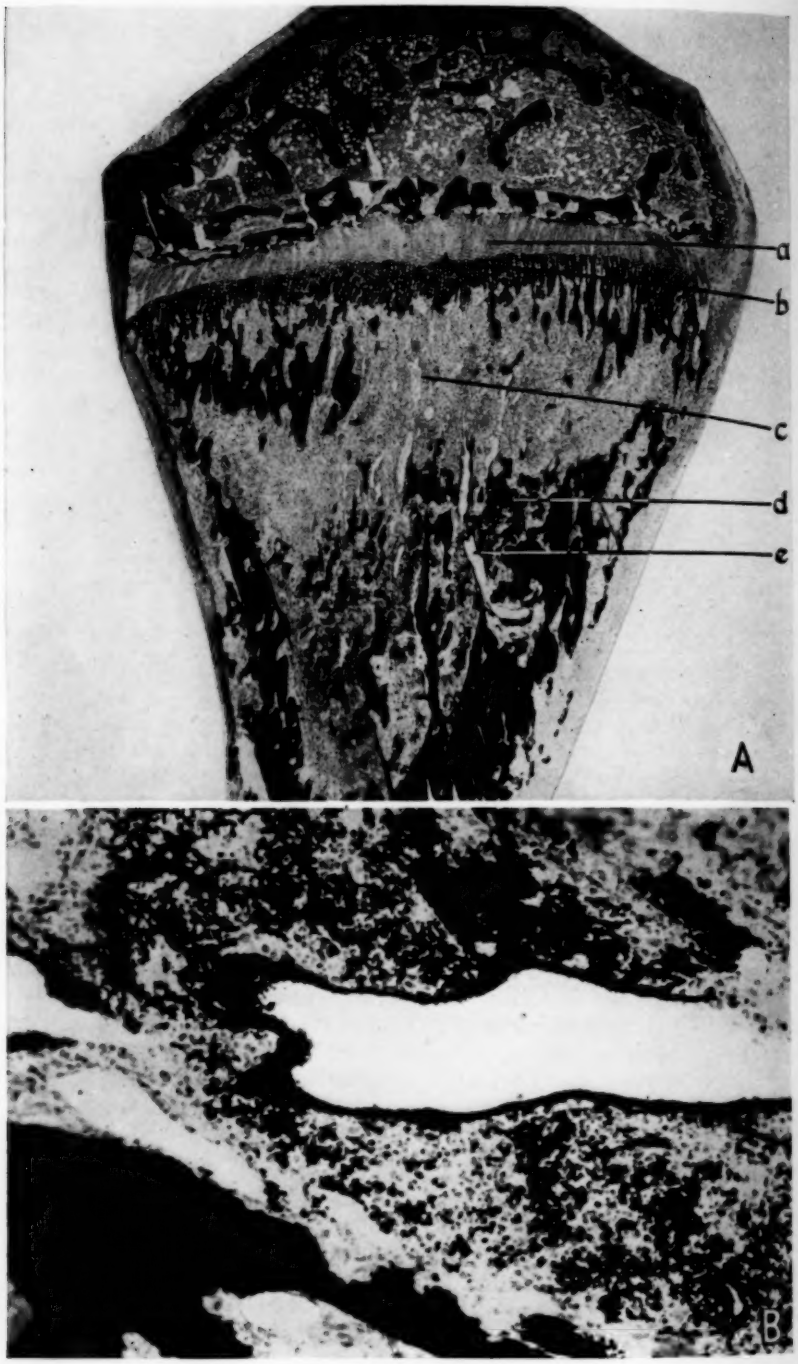

Figure 4

(See legend on opposite page)

network and as aggregations within phagocytic cells. Figure 5 illustrates osteoclasts in contact with disintegrating bone. These cells have an occasional crystal of the silver salt in association with them but no aggregation of the mineral or of debris within them, and the boundaries between them and the bone are more sharply defined than in the similar material from puppies illustrated in figure 2. Figure 5 *B* illustrates also bone salt aggregated in macrophages; the network in which crystals are deposited is seen as a background to the intercellular crystals.

Rats 10901-10908.—A second experiment was undertaken in an effort to duplicate the conditions of the experiment just described, with the addition of 2 rats that continued on the normal diet while receiving parathyroid extract. Only 1 rat in the series, no. 10906, which received 600 units of parathyroid extract in seventy-two hours while on the low phosphorus diet, responded with a moderate amount of demonstrably mobilized bone salt. The findings in sections from this rat were similar to those just described, but slightly less advanced. There were necrosis in the marrow, resorption of bone and early osteitis fibrosa, and the silver salt in the marrow was in part aggregated within phagocytic cells and in part deposited in a basophil network. In sections from the rats examined at eighty-four and one hundred and two hours, especially the latter, the evidences of healing, with a return of osteoblastic activity and the formation of new bone, predominated, and no mobilization of bone salt was demonstrable.

Rats 9601-9604.—This was a litter of younger rats, weaned to fox chow at the age of 21 days and 27 days old when killed. Sections from 2 animals, nos. 9603 and 9604, which received 200 and 400 units of parathyroid extract in one and two days, respectively, exhibited demonstrable mobilization. Two other animals in the litter died during the administration of the extract and were not examined. This experiment is of particular interest in that tissues from the animals were examined also after fixation by Dr. I. Gersh with the Altmann-Gersh freezing-drying technic,[9a] by means of which the possibility of postmortem movement of bone salt is reduced to a minimum.

The formaldehyde-fixed material from rats 9603 and 9604, impregnated with silver nitrate and counterstained with hematoxylin and eosin, showed considerable amounts of black-staining salt in the marrow. Salt not in cells was present in the form of crystals, with a background of purple-staining granular substance. In the frozen-dried material, as described by Bloom and Bloom[9] for embryonic bone, the bone salt was dark brown, finely granular and more evenly distributed in the network, almost completely obscuring the substance in which the salt was deposited.

EXPLANATION OF FIGURE 4

A, undecalcified tangential section through the head of a tibia of rat 6304 after the animal had received 600 units of parathyroid extract in seventy-two hours (for comparison with normal bone, see figure 1, McLean and Bloom[1]); (*a*) epiphysial cartilage; (*b*) zone of provisional calcification and primary substantia spongiosa; (*c*) zone of completed resorption, free from bone salt; (*d*) mobilized bone salt diffusely permeating the bone marrow; (*e*) blood vessel, shown in higher power in *B*. Formaldehyde fixation; 10 microns; silver nitrate, hematoxylin and eosin; photomicrograph, × 22.

B, higher power view of venule shown in *A*, at *c*. There is diffuse permeation of the marrow with bone salt and dense deposit of the salt in the endothelium of the venule. Photomicrograph, × 201.

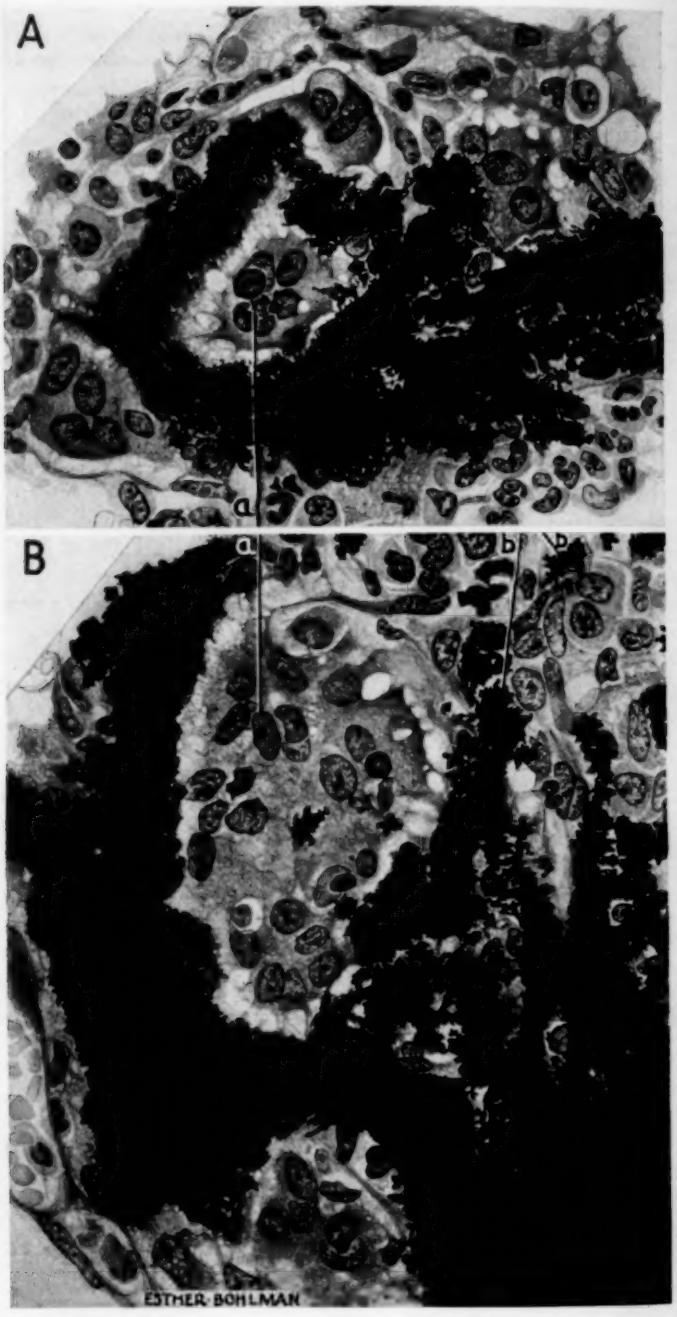

Figure 5

(See legend on opposite page)

The salt aggregated in phagocytic cells was also in a more finely granular form in the frozen-dried preparations. In both the frozen-dried and formaldehyde-fixed preparations from rat 9604 there were areas in which considerable amounts of silver were present in marrow cells of ail varieties. In the formaldehyde-fixed preparations the silver was in dense crystals. In the frozen-dried material it was associated with the nuclei, giving them a speckled appearance. In some cells this appeared to be due to precipitation of the mineral on chromatin particles; in others it seemed to have been due to their accumulation within the nuclei in the interstices between ice crystals. This distribution of the silver has not been observed in any other animals, and its significance is not known.

COMMENT

The observations here reported reveal bone salt in the bone marrow, apparently interrupted in transit from the trabeculae to the venules. We have not been able to demonstrate the reverse process, that of bone salt passing through the marrow in the course of calcification of bone. The difficulty in demonstrating the bone mineral depends on the fact that the method employed, impregnation with silver nitrate, is not sufficiently sensitive to demonstrate dissolved bone salt even in considerably supersaturated solutions, but stains the salt only when this is in particulate form. The conditions for demonstration of the salt, therefore, are the conditions for its deposition in solid form. These conditions include saturation of the intercellular fluids with the ions of the salt, and usually also the presence of a calcifiable substrate. The later agregation of the salt within macrophages is discussed later in this comment.

The nature and source of the calcifiable substrate are of great interest. We have shown that it consists of a granular or amorphous substance, staining purple with hematoxylin and similar in appearance to the very early bone matrix in embryonic bone formation.[10] We believe that it is not the tissue fibrin abundant in the necrotic bone marrow of these preparations and that it is not the collagenous fibers of developing bone. It was not present in the bone marrow of the control animals but appeared in both puppies and rats under the conditions of our experiments. It is present in the marrow between the venules and the trabeculae of bone undergoing resorption, and it is not present in

10. Hess, A. F.; Weinstock, M.; Rivkin, H., and Gross, J.: Proc. Soc. Exper. Biol. & Med. **27**:140, 1929.

EXPLANATION OF FIGURE 5

A and *B*, undecalcified sections through the head of a tibia of rat 6305 after the animal had received 800 units of parathyroid extract in ninety-six hours: (*a*) osteoclasts in contact with disintegrating bone, with an occasional crystal of bone salt but with no aggregation of the salt; (*b*) macrophages packed with bone salt. Formaldehyde fixation; 10 microns; silver nitrate, hematoxylin and eosin; camera lucida, × 681.

areas from which resorption of bone has been completed. In view of these facts we have concluded that the probable origin of the substance, like that of the bone salt, is from the dissolving bone. Nothing is known regarding the nature of the substance which confers the property of calcifiability on the matrix of bone and of cartilage, but if such a substance were dissolved from bone during the process of resorption, and if it were deposited in the intercellular spaces of the marrow, where it might serve as a substrate for the deposition of bone salt, it would account for the histologic observations here described.

In a later stage considerable amounts of the salt are found aggregated within cellular elements of the marrow, especially the macrophages and especially those in regions in which the salt had been deposited in the intercellular network. This aggregation of the salt is evidence that it was in particulate form when taken up by the cells. As is well known, colloidal dyes introduced into the circulation are stored in macrophages, including those of the bone marrow, and Gersh[11] found that colloidal calcium phosphate formed in or introduced into the blood stream is taken up by the phagocytes of the liver and spleen. Consequently, a possible interpretation of the aggregation of the salt in macrophages is that it was in colloidal suspension in the tissue fluids. On the other hand, Shipley and Macklin,[12] without having demonstrated ingestion of bone salt by the macrophages of the marrow, expressed the opinion that these cells have the function of taking up the debris incident to resorption of bone and thus helping to clear it away. Our observations are compatible with this suggestion if it is assumed that the mineral-containing network we have described is treated as a foreign body and that its phagocytosis, including that of the mineral laid down in it, represents a part of the process of recovery from the acute effects of overdosage with parathyroid extract.

Shipley and Macklin[12] found that colloidal dyes introduced into the circulation by way of the peritoneal cavity were not found in osteoclasts, although they appeared in the macrophages of the bone marrow. Our observations, added to theirs and to others, appear to absolve osteoclasts of any phagocytic function in the resorption of bone. The assumption of such a function has rested on relatively weak evidence, reviewed by Arey[13] and by Weidenreich.[14] The contrast between the aggregation of bone salt by the macrophages and the absence of such aggregation

11. Gersh, I.: Anat. Rec. **70**:331, 1938; Am. J. Physiol. **121**:589, 1938.

12. Shipley, P. G., and Macklin, C. C.: Am. J. Physiol. **42**:117, 1917.

13. Arey, L. B.: Am. J. Anat. **26**:315, 1920.

14. Weidenreich, F.: Das Knochengewebe, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol. 2, pt. 2, p. 464.

within the osteoclasts, frequently seen in the same microscopic field (figs. 2*B* and 5*B*), is too striking to permit us to believe that the osteoclasts remove mineral from bone by a phagocytic process. Moreover, in the presence of active resorption and extensive necrosis, accompanied by active phagocytosis of cellular debris by macrophages, we have never seen such debris within the osteoclasts. If one concludes that the bone salt or other products of the dissolution of bone pass through the osteoclasts, with or without the active intervention of these cells, one must assume either that these substances remain in solution within the cytoplasm of the cells or that the cells dispose of particulate matter far more rapidly than do the macrophages.

The observations reported here throw some light on, but do not answer, the fundamental question of how the parathyroid hormone so affects bone as to cause it to be dissolved or resorbed. They do not support the theory of Jaffe [15] that bone to be resorbed is first decalcified by simple solution of the bone salt in the tissue fluids and that osteoclastic resorption of the organic matrix follows as a reaction to decalcification. In all specimens of bone which we have studied with attention to the process of resorption, including normal bone,[1] embryonic bone,[9] bone being reorganized in the healing of fractures,[16] medullary bone of laying birds, which shows extremely rapid resorption,[17] and bone under the influence of parathyroid extract, as here reported, we have found that organic matrix and bone salt are resorbed simultaneously. The occasional finding of a fragmented purple-staining bone matrix containing discrete crystals that stain black with silver nitrate, as described in an earlier paragraph (puppy 8103), twelve hours after the administration of parathyroid extract apparently represents the last stage of disintegration of bone with aggregation of the remaining bone salt into crystals during fixation and dehydration.

Other variants of the general theory that the parathyroid hormone acts on bone by increasing the solubility of the bone salt in the plasma and tissue fluids are upheld by Albright and Ellsworth,[18] by Greenwald and Gross [19] and by Shelling.[20] In these reports the histologic changes in the bones, so far as they are considered at all, are regarded as secondary to the solution of the bone salt. Additional direct evidence will be brought against the theories in this category in a subsequent

15. Jaffe, H. L.: Arch. Path. **16**:63 and 236, 1933.

16. Urist, M. R., and McLean, F. C.: J. Bone & Joint Surg. **23**:1, 1941.

17. Bloom, M. A.; Bloom, W.; Domm, L. V., and McLean, F. C.: Anat. Rec. **78** (supp.):143, 1940.

18. Albright, F., and Ellsworth, R.: J. Clin. Investigation **7**:183, 1929.

19. Greenwald, I., and Gross, J.: J. Biol. Chem. **66**:217, 1925.

20. Shelling, D. H.: The Parathyroids in Health and in Disease, St. Louis, C. V. Mosby Company, 1935, p. 211.

paper, in which it will be shown that resorption of bone, with its contained bone salt, continues under the influence of parathyroid extract long after the plasma, and presumably the tissue fluids, have become supersaturated with the salt. In the light of these observations the mobilization here described is best explained by assuming that the bone matrix and its contained bone salt are simultaneously dissolved by a local cellular action, which is not typical phagocytosis by osteoclasts. Such a theory has been advanced by Thomson and Collip [21] and supported by the observations of Selye,[22] who attributed the resorption to the osteoclasts without specifying the nature of their activity.

There remains the possibility that the bone salt deposited in the bone marrow, including that taken up by the macrophages, is derived from the blood, rather than directly from the local or adjacent disintegrating bone, in which case the process would resemble that of pathologic calcification in the other tissues. Such an explanation of the observations reported here would involve the assumption that the bone salt leaves the bone in an unsaturated and returns in a saturated solution. Such an assumption has been made by Ham and Portuondo [23] to explain the late appearance of pathologic calcification after a single large dose of vitamin D. Their view is that the parathyroid hormone in some way holds increased amounts of calcium salts in solution but that as the hormone leaves the blood the plasma is left in a supersaturated condition and deposits its excess of the salts. This view is not compatible with our demonstration, earlier referred to, that the plasma is actually supersaturated with the bone salt during the resorption of bone. Moreover, the phenomenon described here is demonstrable much earlier than pathologic calcification has been shown to occur following administration of parathyroid extract. Our observations on the possibility of pathologic calcification in our animals are not complete, but we have no indication that the deposition of calcium salts in the bone marrow is coincident with a similar process elsewhere in the body.

Finally it should be stated that all of the observations reported in this paper refer to toxic doses of parathyroid extract and that no attempt has been made, on the basis of these findings, to contribute to the understanding of the mode of action of the parathyroid hormone within the physiologic range. Our contributions on this phase of the subject will be published separately.

SUMMARY

Bone salt, mobilized from trabeculae of spongy bone under the influence of large doses of parathyroid extract and in transit to the venules

21. Thomson, D. L., and Collip, J. B.: Physiol. Rev. **12**:309, 1932.
22. Selye, H.: Endocrinology **16**:547, 1932.
23. Ham, A., and Portuondo, B. C.: Arch. Path. **16**:1, 1933.

of the marrow, has been demonstrated histologically in bones of puppies and rats.

Inasmuch as the method used, i. e., impregnation of undecalcified material with silver nitrate, brings bone salt to visualization only when the latter is in solid form, the conditions for demonstration are those for its deposition and aggregation. These conditions are saturation of the tissue fluid with the ions of the salt and, as a rule, the presence of a calcifiable substrate. The bone salt is also aggregated within the macrophages of the marrow.

During resorption of bone under the influence of toxic doses of parathyroid extract a calcifiable substrate, not present in the marrow of control animals, appears in the intercellular spaces of the marrow, in the form of a granular or amorphous network staining purple with hematoxylin. It is suggested that this may be, or may contain, a specific substance which adds the property of calcifiability to collagenous fibers and that it is deposited in the marrow as a result of the dissolution of the bone in which it had been present. Mobilized bone salt is diffusely deposited in this intercellular substance, where it is demonstrable by impregnation with silver nitrate.

Aggregation of the bone salt within the macrophages of the marrow occurs at a stage somewhat later than that of its diffuse deposition in the intercellular spaces. The taking up of the salt by the macrophages may be phagocytosis of a colloidal complex of calcium and phosphate or may represent a part of the process of recovery from the acute toxic effects of parathyroid extract on bone. During recovery from these toxic effects, the macrophages also take up debris from areas of necrosis in the bone marrow and presumably from disintegrating bone matrix. There is no evidence in our material for similar phagocytic functions by osteoclasts.

During the rapid resorption of bone under the influence of large doses of parathyroid extract, organic bone matrix and its associated bone salt are resorbed simultaneously. There is no evidence from this or from similar material in our hands that resorption of bone matrix occurs as a reaction to its decalcification.

TISSUE REACTIONS IN FATAL CASES OF STREPTOCOCCUS HAEMOLYTICUS INFECTION

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One of the striking features of infections with the hemolytic streptococcus is the occurrence of nonsuppurative lesions which appear at varying periods of time after the onset of infection. They have been referred to as *Nachkrankheiten* by various German investigators,[1] and they include lymphadenitis, nephritis, synovitis, skin eruptions and fever; rarely, endocarditis, hepatomegaly and splenomegaly may be encountered. In addition, one may observe transient hypertension with or without edema and heart failure, or edema alone without other signs of nephritis or cardiac insufficiency. Transient hematuria with albuminuria and cylindruria, as well as alterations in the electrocardiogram, may be observed. All of these features may occur in various combinations or alone, and they suggest that widespread tissue reactions with varying degrees of intensity follow hemolytic streptococcic infections. In many cases these phenomena have been observed following streptococcic infections which produce the clinical picture of scarlet fever, but their occurrence following other streptococcic infections, which are not accompanied by a characteristic skin eruption, is also frequent.

In addition to the aforenamed conditions, it is now well recognized that hemolytic streptococcic infections are not infrequently followed by typical attacks of rheumatic fever, and this is especially true when the subject has had previous attacks of this disease.

The genesis of the various tissue reactions that accompany the clinical features mentioned in the foregoing paragraph has been the subject of numerous investigations. In 1907 Schick [1b] and later, in 1912, Escherich and Schick [1c] called attention to the close similarity between many of the clinical features of serum sickness and the *Nach-*

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1. (*a*) von Pirquet, C.: Ergebn. d. inn. Med. u. Kinderh. **5**:459, 1910. (*b*) Schick, B.: Jahrb. f. Kinderh. **65**:132, 1907. (*c*) Escherich, T., and Schick, B.: Scharlach, Vienna, Alfred Hölder, 1912.

krankheiten of scarlet fever. Their observations led them to postulate that the *Nachkrankheiten* of scarlet fever were due to reactions in the tissues resulting from the development of a state of hyperergia to the streptococcus. This hypothesis has attracted widespread attention, and most investigators who have studied these cases have attempted to explain the appearance of these nonsuppurative features on a basis of tissue reactions which are a response to previous coccal infection.

It was our aim in this study to obtain as thorough a knowledge as possible of the histologic changes occurring in the various tissues during scarlet fever and other types of streptococcic infection. We wished to determine whether these reactions were identical in all streptococcic infections or whether they were in any way specific.

In addition, we wanted to throw some light on the origin and cause of these reactions and, by careful clinical correlation, gain some impression as to their clinical age, their relation to the presence or absence of septicemia, their occurrence in the different age groups of patients, and whether any relation to antibody formation could be determined. Finally, we wished to compare the findings in rheumatic fever with those in the streptococcic infections.

MATERIAL

The necropsy material was obtained from the Mallory Institute of Pathology, and the clinical information, from personal observations and the records of the Boston City Hospital. Ninety cases of fatal streptococcic infection were studied. Of these, 47 were cases of scarlet fever; 26, erysipelas, and 17, hemolytic streptococcic sepsis originating from cellulitis, puerperal infections or septic wounds. Routine histologic sections of all the organs were available in the majority of the cases, but in most instances only the heart and the kidney were studied in detail.

HISTOLOGIC CHANGES

Heart in Streptococcic Infection.—In cases of scarlet fever, by far the commonest lesions observed in the heart consisted of foci of lymphocytes, plasma cells and histiocytes. These were most numerous in the endocardium, lying in the connective tissue just beneath the endothelium. They could occur anywhere along the endocardium but seemed to have a definite tendency to be most common in the regions somewhat protected from the full force of the cardiac blood stream, such as that behind a papillary muscle or that extending up along the thebesian veins. Such lesions are illustrated in figures 2, 5 and 6. They are small, measuring 0.2 mm. or less in diameter.

Similar lesions in the myocardium and pericardium, although perhaps less conspicuous, could practically always be found in cases in which any marked degree of involvement was shown. Here again they were definitely focal rather than diffuse. In the myocardium they were most frequently found in the perivascular connective tissue, but small foci

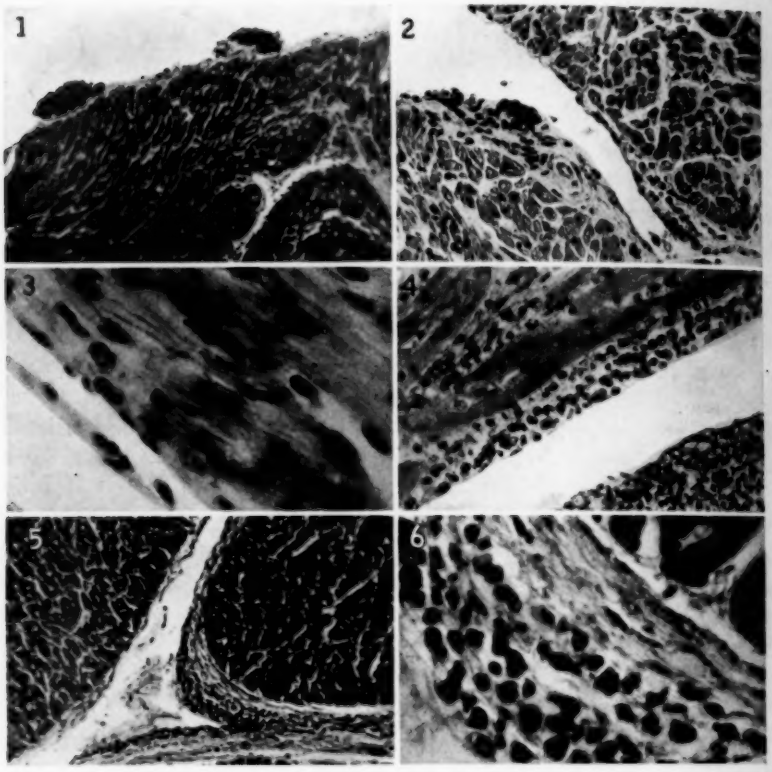


Fig. 1.—Heart showing small thrombi attached to the endocardium. These thrombi consist of fibrin and polymorphonuclear leukocytes. In some thrombi of this type rare gram-positive cocci can be demonstrated. The patient died of an overwhelming streptococcic infection twelve hours after giving birth to a child. Phloxine–methylene blue stain; × 226.

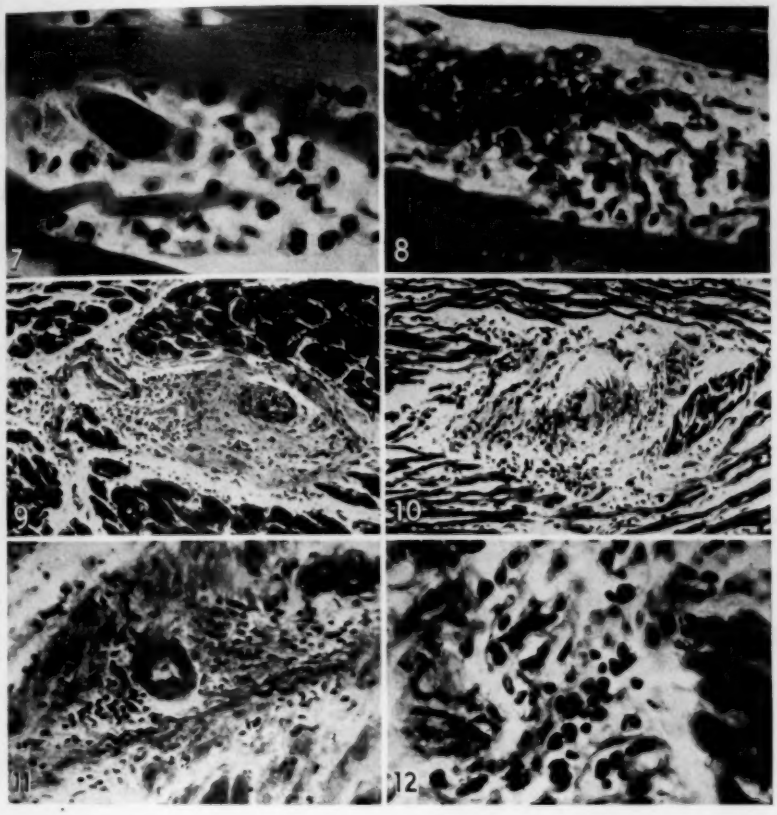
Fig. 2.—Heart from a patient who died of scarlet fever. Foci of lymphocytes, plasma cells and histiocytes are seen in the connective tissue just beneath the endothelium. Phloxine–methylene blue stain; × 378.

Fig. 3.—Heart from a child who died of streptococcic sepsis complicating a burn. Several cocci can be seen in the connective tissue underlying the endothelium. MacCallum-Goodpasture stain; × 1417.

Fig. 4.—Heart from a child who died of scarlet fever. A cellular focus beneath the endothelium contains not only lymphocytes and plasma cells but many polymorphonuclear leukocytes. Phloxine–methylene blue stain; × 755.5.

Figs. 5 and 6.—Heart from a patient who died of scarlet fever, showing the typical location and composition of the usual endocardial lesions. A cellular focus lies in the endocardium and is composed of plasma cells, lymphocytes and rare histiocytes. Phloxine–methylene blue stain; figure 5, × 566.6; figure 6, × 1417.

also occurred between the muscle fibers with no apparent connection with the connective tissue. In the perivascular lesions, the arterial walls were not involved, but the infiltrating cells were distributed around the veins and lymphatics. Two such lesions are shown in figures 11 and 12. The foci were most common in regions adjacent to the endocardium and the pericardium, and sometimes one could trace direct continuity between



Figs. 7, 8, 9 and 10.—Hearts from patients dying of septic sore throat with blood cultures positive for Str. haemolyticus. Phloxine–methylene blue stain.

Figure 7 shows a blood capillary or lymphatic filled with cocci which have escaped into the surrounding tissue and which have given rise to an exudation of polymorphonuclear leukocytes. × 1417.

Figure 8 shows an interstitial focus consisting of fibrin, polymorphonuclear leukocytes and rare lymphocytes. × 755.5.

Figures 9 and 10 show perivascular lesions consisting chiefly of polymorphonuclear leukocytes. × 566.6.

Figs. 11 and 12.—Sections from the same heart as those shown in figures 5 and 6. A perivascular cellular focus consisting mostly of lymphocytes and plasma cells is shown. Phloxine–methylene blue stain; figure 11, × 566.6; figure 12, × 850.

the myocardial lesions and those in the adjoining structures. This extension was almost invariably along the course of large or small blood vessels.

The pericardial lesions were very similar to those described in the endocardium and myocardium; that is to say, they were focal, tended to surround veins and lymphatics and consisted of the same type of cellular reaction.

Although, as has been described in a foregoing paragraph, the usual cellular composition of these foci in cases of scarlet fever was mononuclear, moderate numbers of polymorphonuclear leukocytes were observed in a fair number of cases, and even rare eosinophils scattered among the mononuclears, suggesting that these lesions might be somewhat more acute than the commoner ones (fig. 4).

In cases of erysipelas and other types of streptococcic infection, identical lesions were found in the heart. Here, however, particularly in the cases of erysipelas, there was a definite tendency for more polymorphonuclear leukocytes to be present in the lesions.

Controls.—As controls, we examined the heart muscles from 91 persons who had died of the following conditions: 33 of syphilitic aortitis, 26 of pneumococcic pneumonia, 26 of infection other than hemolytic streptococcic infection (all children under the age of 10 years), 12 of streptococcic endocarditis and 20 of acute rheumatic fever.

In the heart in the cases of syphilitic aortitis it was not uncommon to find foci of lymphocytes in the pericardium, and in 2 cases there were perivascular areas of fibrosis with a few histiocytes and plasma cells collected in these areas. Otherwise, the heart muscle was without pathologic change.

In several cases of lobar pneumonia small foci of lymphocytes were found in the endocardium and pericardium, and in 2 cases there were a few perivascular foci of histiocytes about the smaller blood vessels in the myocardium. Generally speaking, the heart muscle showed no striking evidence of an inflammatory process.

In the group of cases of nonstreptococcic infection in children under 10 years of age there was only 1 case in which an occasional perivascular collection of histiocytes was found.

From the results of the cardiac examinations in the aforementioned three groups, numbering 85 cases, it can be concluded that in the types of infection represented interstitial reactions were infrequent and never as conspicuous a feature as they were observed to be in the cases of fatal hemolytic streptococcic infection.

In the 12 cases of Streptococcus viridans endocarditis, the heart showed a much more complicated picture. Vegetations were found on the heart valves, chordae tendineae and, in some cases, on the auricular

wall. In 2 of these cases definite and typical Aschoff bodies were present. In others miliary areas of necrosis with infiltration by polymorphonuclear leukocytes were found. These were probably embolic in origin. In addition to these findings a reaction very similar to, if not identical with, that already described as typical for hemolytic streptococcic infection was present. In 8 cases foci of lymphocytes, plasma cells and occasional polymorphonuclear leukocytes occurred in the endocardium. These were frequently located around the openings of the thebesian veins and varied in number from an occasional one to several in a single high power microscopic field. In some of these cases perivascular foci of histiocytes and plasma cells were found in the interstitial tissue.

Heart in Acute Rheumatic Fever.—Histologic sections of the myocardium were studied in 20 cases of acute rheumatic heart disease. The only criterion used in their selection was that they must contain typical Aschoff bodies showing both necrosis of collagen and Aschoff giant cells.

The distribution of the Aschoff bodies was found to be very similar to that of the lesions in the cases of streptococcic infection. They were common beneath the endocardium, perivascularly and interstitially. Aschoff bodies were never found in the epicardial fat, whereas in the cases of streptococcic infection foci of infiltration were common in this fat. In the case of rheumatic fever, however, this same type of infiltration was common.

When one compared the lesions found in the myocardium in these two conditions as to cellular composition, they were very similar except for the fact that the Aschoff body contained the Aschoff giant cells and also necrotic-appearing collagen at its center. Around all the Aschoff bodies peripherally were varying numbers of lymphocytes, plasma cells and histiocytes. Also in the rheumatic hearts foci of these cells occurred without any of the more specific elements being found. Connective tissue with dense collagen was much more prominent in rheumatic hearts than in those involved in streptococcic infection, and the Aschoff bodies seemed to occur predominantly in these regions of scarring.

Kidneys.—Kidneys from persons who have died of scarlet fever, as has been known for a long time, frequently show what is called interstitial nephritis. This consists of focal areas of infiltration in the interstitial tissue surrounding the glomeruli, tubules and blood vessels. The process is most marked in the cortex but may extend to the pyramids. This was essentially the picture that we found in many of our cases of scarlet fever and streptococcic sepsis. The common lesion consisted of focal areas of infiltration in the interstitial connective tissue, made up for the most part of lymphocytes and plasma cells. As was also true of the heart, a few to a moderate number of polymorphonuclear leukocytes might

be found among the infiltrating cells. The glomeruli and the tubules showed no consistent pathologic alteration. The changes are illustrated in figures 14, 15 and 16. Figure 14 is a low power photomicrograph of a kidney in a case of scarlet fever to show the focal character of the infiltration. Figures 15 and 16 are higher power photomicrographs of

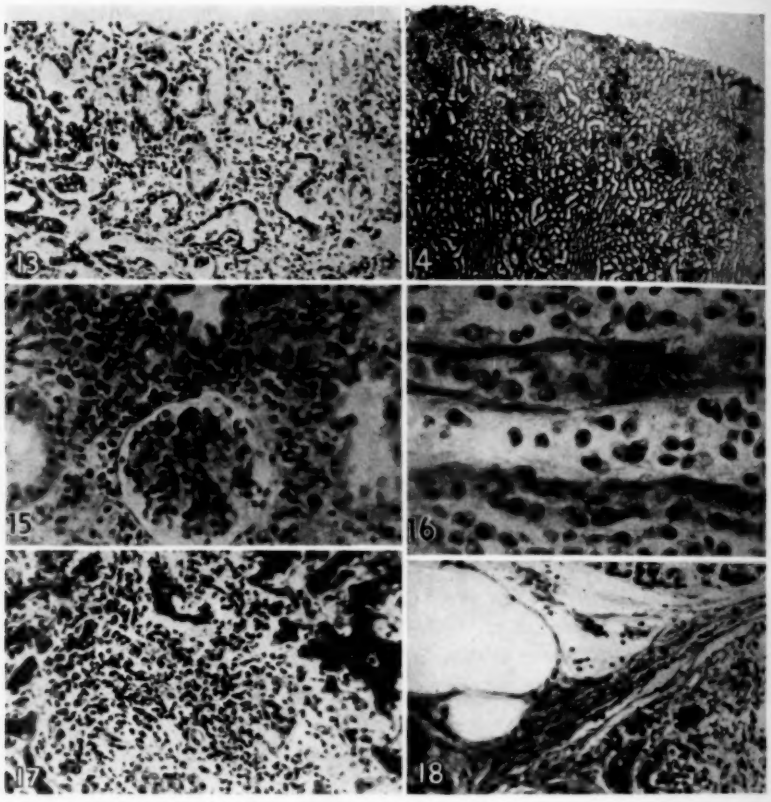


Fig. 13.—Kidney from a patient who died of streptococcic sepsis. An interstitial reaction of polymorphonuclear leukocytes is seen. Similar cells are also present in the lumens of some of the tubules. Phloxine-methylene blue stain; × 755.5.

Fig. 14.—Low power photomicrograph of a kidney from a patient who died of scarlet fever, showing the focal distribution of the interstitial reaction. Phloxine-methylene blue stain; × 47.2.

Figs. 15 and 16.—Kidneys from patients who died of scarlet fever. A typical interstitial reaction of lymphocytes, plasma cells and histiocytes is seen. Phloxine-methylene blue stain; figure 15, × 566.6; figure 16, × 755.5.

Fig. 17.—Liver from a patient who died of septic sore throat. Note infiltration of the portal area by polymorphonuclear leukocytes and rare lymphocytes. Phloxine-methylene blue stain; × 566.6.

Fig. 18.—Pancreas from the same patient as the liver shown in figure 17. Acute interstitial pancreatitis is shown by a reaction of polymorphonuclear leukocytes in the interstitial connective tissue. Phloxine-methylene blue stain; × 614.

areas of infiltration in similar kidneys. The infiltrating cells can be seen to lie in the interstitial connective tissue. Not infrequently polymorphonuclear leukocytes could be found in the lumens of the tubules (fig. 13). This was most common in those cases in which these cells were moderately numerous in the interstitial tissue.

Other Tissues.—Cellular reactions of the type already described did not occur only in the heart and kidneys. Similar processes could be demonstrated in many of the other organs. In cases of scarlet fever the lungs had areas of infiltration around the bronchioles and, to some extent, in the pleura and fibrous septums. The spleen had large eccentrically placed foci of plasma cells and lymphocytes beneath the intima of the veins. Similar cells were observed in the pulp and beneath the capsule. The same type of infiltration was found in the portal areas of the liver (fig. 17), in the fibrous tissue around the ducts of the pancreas (fig. 18) and adjacent to the central vein or in foci between the cortical cells in the adrenals. Even in the pia-arachnoid of the brain a minor degree of infiltration was observed occasionally. Identical lesions could be found also in the organs of patients who had died of erysipelas or other types of streptococcic sepsis.

CHARACTER OF TISSUE REACTIONS IN THREE PATIENTS WHO DIED OF FULMINATING STREPTOCOCCIC INFECTION AND COMPARISON OF THESE WITH THE COMMONER ONES

The findings in 3 cases of acute and fulminating hemolytic streptococcic infection will be described in detail since we believe that these lesions may shed some light on the origin of the commoner and apparently later ones.

The first case was that of an infant, 25 days old, who died three days after the development of streptococcic cellulitis of the leg and buttock. At autopsy little was found save the local septic process. The postmortem blood culture was positive for Streptococcus haemolyticus. Histologically the case was of great interest because there was striking evidence of an acute and overwhelming septicemia. Sections prepared with the MacCallum-Goodpasture stain revealed gram-positive cocci in clumps and chains in blood vessels throughout the body. There was practically no cellular reaction to these organisms, but their distribution was strikingly similar to the distribution of the lesions so commonly found with streptococcic infection.

In the heart, micro-organisms were found in large numbers in a blood clot adherent to the endocardium. In places the organisms occurred in the endothelial cells of the endocardium and had invaded the underlying connective tissue. This is shown in figure 3. Rare organisms were found in the connective tissue of the perivascular areas of the myocardium. Clumps of organisms were also present in the lymphatics

accompanying the blood vessels of the pericardium. When one examined the other organs, the peribronchial lymphatics of the lung, the lymphatics of the pancreas and the adrenals and those in the portal areas of the liver were found to contain large numbers of cocci. In the splenic pulp, many organisms were found which had undergone phagocytosis by the endothelial cells of the blood sinuses. Phagocytosis had also occurred in the Kupffer cells in the liver, and large clumps of organisms were found in the sinusoids.

In view of the lack of reaction to these organisms, the objection might be raised that their distribution could be explained on the basis of postmortem spread. The autopsy was done five hours after death, and the histologic preservation of the tissues was excellent. It seems fair, then, to assume that while it is possible that the organisms increased in numbers after death, the distribution was probably the same ante mortem and that with marked hemolytic streptococcic septicemia diffuse lymphangitis of the various organs may occur and that phagocytosis of cocci by the reticuloendothelium takes place. The distribution also suggests that the organisms can penetrate the endothelium and invade the underlying tissue.

The precise distribution of the organisms in the kidneys was somewhat difficult to determine. Clumps of bacteria were found in the capillaries of the glomerular tufts. They were present also in the interstitial tissue, probably in blood capillaries but possibly in lymphatics.

The second case was that of a woman in whom chills and fever developed just before the birth of a full term child. Her death occurred within twelve hours after the onset of the illness. Postmortem blood cultures contained hemolytic streptococci, and organisms were demonstrated in the blood vessels of many organs. The heart was of considerable interest histologically. Numerous small thrombi were found attached to the endocardium. They were microscopic in size and consisted of fibrin, polymorphonuclear leukocytes and rare gram-positive cocci (fig. 1). The subendothelial connective tissue showed no reaction. The distribution of these thrombi was quite similar to the foci of lymphocytes and plasma cells already described as the more common type of reaction found in the endocardium in cases of streptococcic infection. Also, in this same heart, rare cocci could be found in the endothelial cells of the endocardium and, in places, in the subendothelial connective tissue.

The third case was of great interest because the condition observed seemed to represent an intermediary stage between those in the two cases just described, in which organisms alone or organisms and thrombi were found, and the more common lesions which have already been discussed. It was that of a woman about 60 years of age, who was admitted to the hospital complaining of a sore throat of three weeks' duration. A diagnosis of septic sore throat was made on admission, and

Str. haemolyticus was isolated from the circulating blood. She died of sepsis and cardiac insufficiency six days after admission. Autopsy showed little grossly aside from signs of acute septicemia.

Histologically, lesions similar in distribution to the common ones were found in the heart and other organs. The type of cellular reaction in these lesions was, however, quite acute in character and consisted of polymorphonuclear leukocytes and fibrin. Also in many of these lesions rare but definite gram-positive cocci could be found when the tissues were stained by the MacCallum-Goodpasture method. Such lesions are shown in figures 7, 8 and 9.

TABLE 1.—*Frequency of Involvement of the Heart and Kidney in 79 Cases of Scarlet Fever, Erysipelas or Str. Haemolyticus Sepsis*

	Cases
Heart involved alone	20
Kidney involved alone	10
Both heart and kidney involved	26
No involvement of either organ	23
Total	79

TABLE 2.—*Incidence and Extent of Reactions Found in Heart in Cases of Streptococcic Infection*

Infection	Cases	Number with Reactions	Per Cent with Reactions	Number with Given Degree of Reaction			
				+	++	+++	++++
Scarlet fever	47	31	65	7	9	8	7
Erysipelas	26	14	53	4	6	3	1
Streptococcic sepsis	17	12	70	3	2	3	4
Total	90	57	63	14	17	14	12

DISTRIBUTION OF THE LESIONS

It was found that lesions might be present in the heart and not in the kidney or vice versa. Of the original 90 cases, satisfactory sections of the heart and kidney were available in 79. The frequency of involvement of these two organs can be seen in table 1.

From table 1 it may be seen that involvement of the heart alone was twice as frequent as involvement of the kidney alone and that involvement of one or the other or both was present in about 70 per cent of the cases. This variation in distribution was also noted by Fahr [2] and Siegmund.[3]

2. Fahr, T.: Beitr. z. path. Anat. u. z. allg. Path. **85**:445, 1930.

3. Siegmund, H.: Centralbl. f. allg. Path. u. path. Anat. **44**:314, 1929.

The incidence and extent of the histologic changes found in the heart following various streptococcic diseases are shown in table 2. For each organ, the degree of involvement was rated on a scale of 4 plus, 1 plus indicating a minimal and 4 plus a maximal number of lesions.

It can be seen in table 2 that examples of slight to severe reaction were found in each of the three groups. These pathologic changes were found slightly more frequently after streptococcic bacteremia following wound infection than after scarlet fever or erysipelas.

A similar summary of the changes in the kidney is shown in table 3. The changes were found most frequently in scarlet fever and less frequently in erysipelas and in wound infection.

TABLE 3.—*Incidence and Extent of Reactions Found in Kidney in Cases of Streptococcic Infection*

Infection	Cases	Number with Reactions	Per Cent with Reactions	Number with Given Degree of Reaction			
				+	++	+++	++++
Scarlet fever	44	24	54	7	7	6	4
Erysipelas	22	8	36	1	5	2	0
Streptococcic sepsis	14	6	42	0	3	1	2
Total	80	38	47	8	15	9	6

TABLE 4.—*Age Distribution of Patients with Fatal Streptococcic Infection*

Decades of Life	Patients
0–10	40
11–20	8
21–30	11
31–40	14
41–50	1
51–60	9
61–70	7

CORRELATION OF THE REACTIONS IN THE TISSUES WITH THE AGE OF THE PATIENT AND THE DAY OF DEATH

The fatality rate from the various forms of hemolytic streptococcic infection in relation to the age of the patient is highest during the first decade and after the age of 50 years.[4] It was not surprising, then, to find that more of the patients in our series were in the first decade than in any other single decade of life. The age distribution is shown in table 4.

In tables 5 and 6 the frequency and intensity of the reactions found in the heart and kidneys are correlated with the age of the patients.

4. Keefer, C. S.; Ingelfinger, F. J., and Spink, W. W.: Arch. Int. Med. **60**: 1084, 1937.

When these tables were analyzed, some interesting facts emerged. First of all, reactions to these infections were more common in the heart than in the kidneys in all decades save the seventh, and in this

TABLE 5.—*Correlation of Degree of Reaction in Heart with Age of Patient*

Decade of Life	Patients with No Reaction	Patients with Given Intensity of Reaction				Per Cent with Changes
		+	++	+++	++++	
0-10	13	6	6	7	8	66
11-20	4	2	2	0	0	50
21-30	0	2	2	4	3	100
31-40	8	1	5	0	0	42
41-50	0	0	0	1	0	100
51-60	3	2	2	2	0	70
61-70	5	1	0	0	1	29
Total	33	14	17	14	12	

TABLE 6.—*Correlation of Degree of Reaction in Kidney with Age of Patient*

Decade of Life	Patients with No Reaction	Patients with Given Intensity of Reaction				Per Cent with Changes
		+	++	+++	++++	
0-10	20	4	6	2	4	45
11-20	4	1	1	2	0	50
21-30	2	1	2	3	2	80
31-40	6	0	2	1	0	33
41-50	1	1	1	0	0	66
51-60	7	0	0	1	0	14
61-70	2	1	3	0	0	66
Total	42	8	15	9	6	

TABLE 7.—*Correlation Between Day of Death and the Intensity of the Reaction in the Heart*

Day of Death	Patients with No Reaction	Patients with Given Intensity of Reaction				Total Number with Changes	Per Cent with Changes
		+	++	+++	++++		
1- 5	15	4	2	0	0	6	29
6-10	14	3	11	7	5	26	65
11-15	2	4	2	3	3	12	86
16-20	1	1	1	2	2	6	86
21-25	1	2	1	1	1	5	84
26+	0	..	..	1	1	2	100
Total	33	14	17	14	12	57	

decade the cases were too few to be of any great significance. Also in both the heart and the kidney reactions were more common under 30 years of age than they were over this age. Under 30 years 72 per cent of the hearts and 50 per cent of the kidneys showed reactions, whereas over 30 years only 50 per cent of the hearts and 30 per cent of the kidneys

reacted. Therefore, the tissues of the younger age groups reacted more to the infection than those of the older ones.

The reactions found in the heart and kidneys were closely correlated with the duration of the disease before death occurred. This correlation is shown in tables 7 and 8. As can be seen in these tables, the duration of the infection is of considerable significance in relation to the occurrence and the intensity of the reaction. It was not until infection had been present for six days that a large proportion of the hearts and kidneys showed changes, and the more marked reactions were never observed under six days. Moreover, the longer the patient lived the

TABLE 8.—*Correlation Between the Day of Death and the Intensity of the Reaction in the Kidney*

Day of Death	Patients with No Reaction	Patients with Given Intensity of Reaction				Total Number with Changes	Per Cent with Changes
		+	++	+++	++++		
1- 5.....	11	1	2	0	0	3	21
6-10.....	23	4	8	3	3	18	45
11-15.....	6	1	3	3	1	8	57
16-20.....	1	1	1	1	2	5	84
21-25.....	1	1	1	2	..	4	80
26+.....	..	..	..	..	..	..	..
Total.....	42	8	15	9	6	38	

TABLE 9.—*Correlation of Changes in Heart and Kidneys with Bacteremia*

	Cases	Number in Which Heart Showed Changes	Number in Which Heart Showed No Changes	Number in Which Kidney Showed Changes*	Number in Which Kidney Showed No Changes*
Bacteremia.....	54	33	21	23	24
No bacteremia.....	12	5	7	3	9

* In the 54 cases with positive blood cultures there were only 47 kidneys available for study.

more apt the heart and kidneys were to show reactions. This is shown graphically in figure 19.

In summary it can be said that patients under 30 years of age and those surviving more than six to ten days showed the greatest number of inflammatory foci and foci of the greatest intensity.

CORRELATION OF LESIONS AND BACTEREMIA

In the late stages the lesions described do not have the appearance of true infectious processes. In rare cases, however, in which death occurred within forty-eight to seventy-two hours, organisms could be demonstrated in the lesions. Therefore, it seemed of interest to determine the incidence of bacteremia at the time of death and to correlate the presence of bacteremia with the tissue reactions. The results are summarized in table 9.

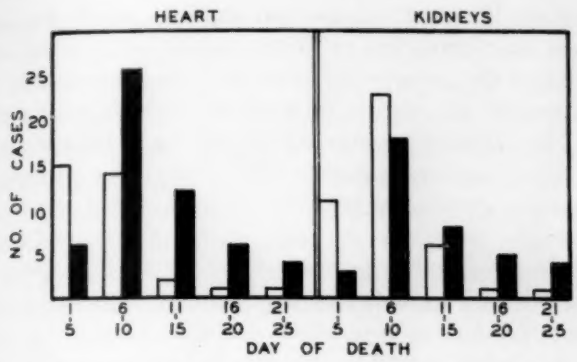


Fig. 19.—Correlation of presence of tissue reactions with time of death. The white columns represent cases in which no changes were observed; the black, cases in which changes were present.

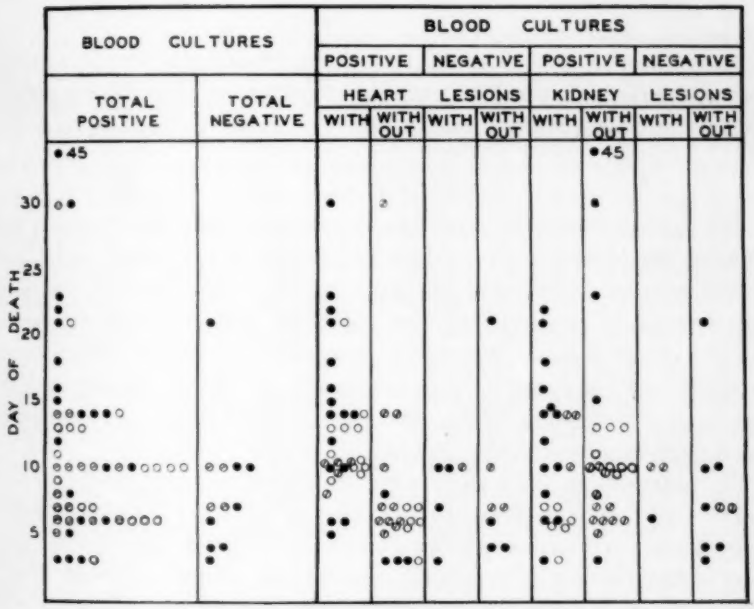


Fig. 20.—Relation of bacteremia to day of death and to presence of tissue reactions in the heart and kidneys. The black circles represent patients who died of scarlet fever; the barred circles, patients who died of erysipelas, and the white circles, patients who died of streptococcic sepsis.

Information relative to bacteremia was available in 66 cases. In figure 20 the relation of bacteremia to the day of death and the presence of reactions in the heart and kidneys had been charted. From this collection of data it was found that in these cases death occurred anywhere from the third to the twenty-third day after the onset of the infection and that bacteremia was present at the time of death in 81 per cent of the patients. The relation between the day of death, bacteremia and the presence of tissue reactions was of interest. Fifty-three patients showed changes in the myocardium at the time of death. Of these, 49 (92 per cent) died on the tenth day or later. It was also noted that of 21 patients with bacteremia who lived less than ten days, 17 (80 per cent) showed no lesions. This indicates that patients with bacteremia who live less than ten days seldom show extensive changes in the heart muscle and that when reactions occur in the heart muscle they do so in patients who have lived longer than ten days. When the cases without bacteremia were studied, the same observation was made; namely, that 5 of the 7 patients showing no lesions died before the tenth day and that only 2 of 5 patients showing lesions died before the tenth day. These results suggest that in cases with bacteremia the presence of tissue reactions in the heart depends at least in part on the duration of the illness.

As to the reactions of the kidney, it was found that they also were observed more often after the tenth day than before, although the difference was much less striking than in the case of the reactions in the heart.

COMMENT

The evidence obtained from the observations described justifies the following statements: The largest number of the patients with fatal hemolytic streptococcic infection were in the first decade of life, and death frequently occurred between the third and the tenth day after the onset of the disease. Bacteremia was present in 80 per cent of those who died. In those dying without bacteremia, diffuse bronchopneumonia, some other complicating disease or a large focus of infection was responsible for the fatal outcome.

The reactions in the tissues were seen most often in the heart and kidneys, and they were present more often in the heart alone than in the kidney alone. We have found, in common with Brody and Smith,[5] and others,[6] that the tissue reactions seen in the spleen, adrenals, liver, gallbladder and other organs are all of the same type.

Reactions in both the heart and the kidneys were more common in patients under 30 years of age, in those living longer than six to ten

5. Brody, H., and Smith, L. W.: Am. J. Path. **12**:373, 1936.
6. Fahr.[2] Siegmund.[3]

days after the onset of the illness and in those with bacteremia at the time of death.

The unequal distribution and the unequal degree of intensity of the tissue reactions may explain in part the variations in the clinical features that one observes following hemolytic streptococcic infections. For example, it is not at all infrequent to see signs of nephritis alone or even signs of heart failure alone, but one may encounter nephritis and electrocardiographic changes indicating heart muscle involvement in this disease; indeed, outspoken signs of heart failure are not infrequent. Or one may have the signs of cardiac disease without any signs of acute nephritis, or there may be minimal changes in the urine. Faulkner, Place and Ohler [7] have found electrocardiographic changes at the tenth to fourteenth day of illness in 6 per cent of all cases of scarlet fever; others [8] have found electrocardiographic changes associated with acute nephritis or, indeed, acute heart failure. It does not seem farfetched, then, to suggest that the variation in the clinical picture observed during the following hemolytic streptococcic infection is due in part to the differences in the intensity and frequency of the reactions in the various tissues.

The precise nature of these tissue reactions found in scarlet fever and other types of streptococcic sepsis cannot be decided from a study of this kind alone. There are certain possibilities, however, that must be considered and that are worthy of comment.

These reactions have been considered by some (e. g., Brody and Smith [5]) as the response of the tissues to a diffuse circulating bacterial toxin. Although this is undoubtedly a possibility, the explanation of the definitely focal character of the lesions by such a hypothesis is difficult.

The acute lesions described by us suggest the possibility that in the earliest stages organisms are present in the lesions and that they first provoke an acute reaction in the form of an exudate of polymorphonuclear leukocytes and fibrin. In the commoner lesions, however, no organisms are found, and the cellular reaction is predominantly mononuclear rather than polymorphonuclear. Nevertheless, foci of infiltration of this type can be explained as a later stage of the earlier lesions. In other words, they can be interpreted as healing lesions in which the organisms have been phagocytosed and destroyed and the acute reaction replaced by a more chronic one.

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7. Faulkner, J. M.; Place, E. H., and Ohler, W. R.: Am. J. M. Sc. **189**:352, 1935.

8. Master, A. M.; Jaffe, H. L., and Dack, S.: Arch. Int. Med. **60**:1016, 1937. Rubin, M. I., and Rapoport, M.: Am. J. Dis. Child. **55**:244, 1938. Whitehill, M. R.; Longcope, W. T., and Williams, R.: Bull. Johns Hopkins Hosp. **64**:83, 1939.

The fact that these lesions are more prominent and occur with greater frequency ten days or more after the onset of the infection is somewhat difficult to explain. There are a number of experiments on animals and man which seem to have a bearing on the mechanisms of tissue reactions following infections. These merit consideration in attempting to explain this feature.

Tissue reactions following injections of bacteria into animals have been studied by many investigators. Special emphasis has been placed on the difference in response between normal animals and those that have been subjected to previous injections of bacteria so that they are in various stages of immunity or hypersensitivity. Nye and Parker,[9] MacMahon and Mallory,[10] Clawson,[11] Swift and his associates [12] and many others have been active in the study of these problems, and there are several excellent summaries available.

Nye and Parker [9] and others have found that rabbits which have received repeated intravenous injections of relatively large doses of dead bacteria or colloidal substances show a marked reaction of the tissues containing cells of the reticuloendothelial system. This reaction consists of an increase in the lymphoid cells, which are transformed into or replaced by monocytes and giant cells. These lesions are of a transient nature and result in no permanent damage. They are interpreted as having nothing to do with the reactions secondary to sensitization or immunization. It should be noted that the reactions are most intense within the first two to four days after the last injection, and when the animals are examined later, the reactions are either absent or minimal. While it appears from these observations that the distribution of the tissue reactions is similar in many respects to that which occurred in the cases we have studied, the reactions were found most intense within the first two to four days after the last injection and not later.

Experiments of a different kind are of interest in connection with our observations. The studies of Andrewes, Derick and Swift [13] and Derick and Andrewes [14] on the secondary reactions in the skin of rabbits that had been given injections of nonhemolytic streptococci or horse

9. Nye, R. N., and Parker, F., Jr.: Am. J. Path. **6**:381, 1930.

10. MacMahon, H. E., and Mallory, F. B.: Am. J. Path. **7**:299, 1931.

11. Clawson, B. J.: Arch. Path. **8**:664, 1929.

12. Swift, H. F., and Derick, C. L.: Proc. Soc. Exper. Biol. & Med. **25**:224, 1927. Swift, H. F.; Hitchcock, C. H., and Derick, C. L.: ibid. **25**:312, 1928. Swift, H. F.; Derick, C. L., and Hitchcock, C. H.: J. A. M. A. **90**:906, 1928; Tr. A. Am. Physicians **43**:192, 1928.

13. Andrewes, C. H.; Derick, C. L., and Swift, H. F.: J. Exper. Med. **44**:35, 1926.

14. Derick, C. L., and Andrewes, C. H.: J. Exper. Med. **44**:55, 1926.

serum are of possible significance in the interpretation of some of the late tissue reactions in man. They found that when rabbits were inoculated intradermally with certain strains of green streptococci, well marked lesions appeared within twenty-four to forty-eight hours and then began to regress, but that in about 50 per cent of them there was a secondary increase in size and other signs of inflammation about eight or nine days after inoculation. At the time of the secondary reaction the lesions were sterile. They brought forth convincing evidence that this secondary reaction was due to a hypersensitive state which was more closely allied to the tuberculin reaction than to the Arthus phenomenon. That is to say, the reaction was the tissue response to some product of the bacterial cell that occurred at the site of previous injection, without any demonstrable humoral antibodies being present. It should be emphasized that this secondary reaction was observed following *one* injection.

It seems not improbable, then, that this reaction was most likely a response on the part of the tissues to the presence of the antigen in the localized area. In the rabbit, however, these secondary reactions were found following the injection of nonhemolytic streptococci and were not observed following that of hemolytic streptococci.

The similarity between the tissue reactions in an experiment of this kind and the secondary tissue reactions in hemolytic streptococcic infection in man is great. This is true with respect to both the time relations and the character of the cellular response.

Now, if the reaction that we are describing is an antigen-antibody reaction, how can the distribution of the lesions and their more frequent appearance after the tenth day be explained?

We have stated previously that the significant lesions were found in the interstitial tissue of the liver, spleen, adrenals, kidney, heart and lymph nodes. To explain the presence of the lesions in these areas, it is necessary to assume that some of the antigen is present here at the time when antibodies are being produced. This has not been demonstrated by us. It has been amply shown that bacteria (i. e., bacterial antigen) are taken out of the circulating blood by the cells of the reticuloendothelial system which are found in the liver, spleen, bone marrow, lymph nodes and adrenals and by the histiocytes about the blood and lymph channels in the interstices of other tissues. Once they are deposited in these areas, they are frequently held there and destroyed. The exact method by which they are killed is not known completely. But that they are disposed of, there is no doubt.[10] Moreover, there is a large body of evidence to show that the organs and cells which are concerned with the clearing mechanism are also concerned in some way with antibody formation, and the antibodies may be present in higher concentration in these tissues during experiments than in the circulating blood or

may even appear in these tissues before one can demonstrate humoral antibodies.[15] There is no clear evidence that any other organs or tissues are responsible for antibody formation. If this is admitted to be the case, it is not farfetched to suggest that the antigen-antibody reaction within tissues is responsible in part for the changes that we have described, especially since they are seen in tissues containing reticuloendothelial cells.

It has been stated previously that organs containing reticuloendothelial cells are concerned in clearing the circulation of bacterial antigen and that reactions in the tissues may take place which have nothing to do with an antigen-antibody reaction. With this in mind, one might urge that it is not necessary to assume that these tissue reactions are of this nature. However, the time of appearance of the lesions is to our mind of the highest importance. The nonantigen-antibody reactions which have been described are most intense within a few days after the infection, whereas the most intense reaction in our cases was observed later. This was about the time when one could demonstrate antibody formation in the patients with hemolytic streptococcic infection.

In many studies it has been found that different antibodies and tissue reactions can be demonstrated at varying periods after the onset of hemolytic streptococcic infection.[16] Many of these antibodies do not appear until after the tenth day, and they occur approximately at the same time as one observes clinical manifestations of tissue changes.

It is now recognized that early in the course of scarlet fever there is a positive skin reaction to the toxin of the organism but that there is little or no reaction to the nucleoprotein fraction of the streptococcus.[17] As the disease progresses and the patient recovers, the Dick test becomes negative and the nucleoprotein reaction becomes strongly positive. The neutralization of the toxin is due to the presence of antitoxin. The nucleoprotein reaction is a hypersensitive reaction in the skin of the delayed type, which has the same histologic characteristics as those described by us in the cases under discussion.[18] This may occur without antinucleoprotein precipitins being present in the blood plasma, and it has not been possible to transfer this reaction passively. It is also known that other streptococcic antibodies appear in the blood, such as antistreptolysins and antibacterial antibodies, in high concentration, about the same time that the nonsuppurative lesions are common.[19]

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15. McMaster, P. D., and Hudack, S. S.: Proc. Soc. Exper. Biol. & Med. **31**:751, 1934. Hudack, S. S., and McMaster, P. D.: ibid. **31**:753, 1934. McMaster, P. D., and Kidd, J. G.: ibid. **34**:547, 1936.
16. Spink, W. W., and Keefer, C. S.: J. Clin. Investigation **15**:21, 1936.
17. Lyttle, J. D.; Seegal, D., and Jost, E. L.: Am. J. Dis. Child. **50**:573, 1935.
18. Spink, W. W.: Arch. Int. Med. **59**:65, 1937.
19. Coburn, A. F.: Internat. Clin. **4**:49, 1936.

From what has been said it appears that there is a fair body of evidence to suggest that late tissue reactions are a response on the part of the host to the infection and that they are the result in part of an antigen-antibody reaction rather than the result of direct toxic damage to the tissues.

It is perhaps significant that suppurative lesions were observed in precisely the same areas as nonsuppurative lesions and that the lesions were found in organs that showed alterations of function following hemolytic streptococcic infections. This is suggestive evidence, at least, that these anatomic lesions are the result of a reaction to the presence of bacterial antigen.

The problem of whether there is any relationship between the lesions found in streptococcic infection and those occurring in rheumatic fever is an important but also very difficult one. Clinically, more and more evidence is being brought forward pointing to a close relationship between these two types of infection.

As has already been described, the microscopic lesions found in these two types of process are alike in many ways but differ in the respect that the two most characteristic features of the Aschoff body, the necrosis of collagen and the occurrence of Aschoff giant cells, are not found in the heart involved in streptococcic infection.

There are two possible ways of interpreting these findings. On the one hand, one can say that the Aschoff body is the characteristic reaction to a specific but as yet unrecognized infectious agent. It could thus be considered just as specific a reaction to this unknown agent as a tubercle is to the tubercle bacillus and the gumma to Spirochaeta pallida. It must be remembered, however, that in spite of the rather characteristic histologic picture found in the tubercle and in the gumma the only faultless proof of their etiologic nature is dependent on the demonstration in them of the specific etiologic agent by means of special stains. This proof is entirely lacking as regards the Aschoff body.

The other possible explanation of the Aschoff body is that it is a later stage of a lesion such as has been described in the heart altered by streptococcic infections. To obtain definite proof for this hypothesis, particularly when one is dealing only with human material, is difficult or impossible. Several features, however, favor this hypothesis. Clinical evidence undoubtedly points to the fact that the duration of the infection is practically always longer in persons with rheumatic fever who come to autopsy than in those with scarlet fever or other types of streptococcic sepsis. In the latter group in our series, death occurred most frequently between ten and fifteen days after the onset of infection. The longest duration of disease was about thirty days. Gross and Ehrlich [20] in an

20. Gross, L., and Ehrlich, J. C.: Am. J. Path. **10**:467 and 489, 1934.

article on a series of cases of acute rheumatic fever described 9 patients, all of whom died in the first acute attack. Death occurred from two to thirteen weeks after the onset of typical symptoms of rheumatic fever, such as pains in the joints and fever. The prodromal period of sore throat or scarlet fever was disregarded in these calculations but was described as preceding the onset by from several days up to four or five weeks. They feel that the earliest specific Aschoff bodies occur two to four weeks after the onset of the symptoms of rheumatic fever. Therefore, it seems justifiable to consider the actual age of the Aschoff body considerably greater than the age of the lesions found in streptococcic infections.

The work of Clawson [11] gives considerable evidence against the specificity of the Aschoff body. He summarized the cardiac findings in 190 cases of chorea or acute rheumatic fever. In all of these careful search for Aschoff bodies was made, but they were found in only 128 (67 per cent). They were not found, therefore, in almost one third of these supposedly typical cases. He pointed out also that Aschoff bodies have been described in cases of nonrheumatic disease. They were found in a case of meningococcic endocarditis by Rhoads.[21]

The frequent occurrence of Aschoff bodies in association with subacute bacterial endocarditis is also of interest. They were found in 27 (45 per cent) of 60 cases by Clawson.[11] They were present in 2 of our 12 cases. These findings are sometimes explained as a superimposed infection of a valve already involved by acute rheumatic fever. If this is true, it is difficult to understand why in these cases joint involvement or symptoms of chorea are rarely, if ever, shown during the illness immediately preceding death.

When one examines this hypothesis, that the lesions of streptococcic infections may be an earlier stage of Aschoff body formation, there is also a certain amount of evidence in its favor from the strictly morphologic point of view. The fate of these streptococcic lesions in the patients that recover is difficult to determine, as autopsy material is not available. It is probably justifiable, however, to suggest that such inflammatory foci may result in some degree of focal fibrosis. When fibrosis occurs, collagen is formed. If continued activity or reinfection occurs, such foci could conceivably result in necrosis of the newly formed collagen, a reaction to this necrotic collagen in the form of giant cells and thus Aschoff body formation.

When one thinks again of subacute bacterial endocarditis, a condition quite ideal for the development of such a process is present. One observes a prolonged infection of the blood stream by a streptococcus. One notes

21. Rhoads, C. P.: Am. J. Path. **3**:623, 1927.

in the heart frequently both types of lesions, those found in streptococcic sepsis and true Aschoff bodies. Because of the prolonged course of the disease it would seem quite justifiable to believe that the earlier lesions might become fibrotic and then become reinfected by the organisms that are still present in the blood stream and, as suggested in the foregoing paragraph, Aschoff body formation would result.

SUMMARY

A study of the various tissues in fatal cases of hemolytic streptococcic infection beginning as scarlet fever, erysipelas, wound infection or puerperal infection showed that anatomic changes were present in the heart, kidneys, liver, spleen, lungs and pancreas. The lesions in the heart and kidneys were studied in detail.

In the heart, the most common lesions consisted of focal accumulations of cells, composed for the most part of lymphocytes and plasma cells. Sometimes polymorphonuclear leukocytes and eosinophils were also present in varying numbers. In rare cases, in which the infection was acute and fulminating, lesions having the same distribution and localization but consisting of organisms alone, organisms plus an acute suppurative reaction, or an acute suppurative reaction alone were found.

The more common lesion, which is apparently the later stage of the lesion just described, was most often seen in patients who died between the sixth and fifteenth days of their illness. The degree of these reactions also was greatest at this time. The reactions were more conspicuous in patients with bacteremia, especially when such patients survived longer than ten days.

From these studies it is suggested that the anatomic lesions were due in part to an antigen-antibody reaction. The anatomic distribution of the lesions also suggests an explanation for the late nonsuppurative phenomena which are not infrequently found clinically following hemolytic streptococcic infections.

A possible relationship between these lesions and Aschoff bodies is also suggested.

"CORRECTED TRANSPOSITION" AND PERSISTENT RUDIMENTARY "RIGHT AORTA" AS EVIDENCE IN SUPPORT OF SPITZER'S THEORY

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The theory of Spitzer [1] has served to make clear many otherwise obscure anomalies of the heart and is receiving increasingly wide acceptance. Since Harris and Farber [2] have recently published an excellent exposition in English of Spitzer's views, no attempt at further explanation will be made here except as relates to certain special features.

Spitzer's contributions include: 1. Emphasis on the role of the advent of the pulmonary circulation during the transition from the aquatic to the terrestrial habitat in causing torsion of the heart.

2. The concept of the role of torsion in the definitive septation of the heart. This results in the crossing of the pulmonic and systemic circulations, thereby allowing full action of a muscular cardiac chamber on each circulation. This is much more efficient than the tandem arrangement in the gill circulation of the fishes.

3. The recognition in man of the rudiment of the right aorta homologous to that of the reptiles and the recognition of the crista supraventricularis as a guide to the position of this rudiment. The crista had previously been homologized by Tandler with the ledge of Greil, which separates the pulmonary artery and the right aorta in the reptiles.

4. The explanation of certain septums as the result of hypertrophy of such structures as the crista supraventricularis, tricuspid ledges or bulboatrial ledge, in association with regressions of the true interventricular septum, on a hemodynamic basis.

5. The concept of detorsion with reopening of the right aorta to explain many anomalies of the heart involving the great vessels.

6. The establishment of four main varieties of transposition based on the degree of detorsion.

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1. Spitzer, A.: Virchows Arch. f. path. Anat. **243**:81, 1923; **271**:226, 1929.
2. Harris, J. S., and Farber, S.: Arch. Path. **28**:427, 1939.

7. The emphasis of the fact that in no case is there a true transposition of any great vessel arising from the right side of the heart to the left if the position of the true interventricular septum is used as the plane of reference.

8. The correlation of the concept of partial situs inversus of the bulboventricular portion of the heart with that of detorsion in its four main types to explain "corrected transposition."

It is obvious that these theories would be put most severely to the test in analyzing the more complex anomalies. In the present study an instance of corrected transposition of the great vessels was subjected to analysis. A description of another heart with an anomaly suggesting persistence of portions of both a right and a left aorta is also presented. If the interpretation of the latter is correct, concrete evidence, not hitherto available, for a fundamental point in Spitzer's theory is at hand.

CASE 1

Origin of bicuspid aorta from anterior position in left-sided ventricle and of tricuspid pulmonary artery from right-sided ventricle; failure of torsion of great vessels; coarctation of aorta; patent ductus arteriosus and foramen ovale; tricuspid left atrioventricular valve; defective false interventricular septum with components from the posterior tricuspid ledge and crista supraventricularis; rudimentary true posterior septum; hypertrophy of right ventricle; coarctation of aorta; acute passive congestion of viscera with central necrosis of liver; multiple strictures of ureters; congestion and hemorrhage of renal medulla.

Clinical History.—A boy was born at term of a healthy mother, aged 24, who had had no previous pregnancies. The delivery was uncomplicated. At birth the infant seemed to be in good condition, and it took its formula well during the first week of life. On the fifth day, however, its temperature was only 95 F., and on the eighth the child seemed weak, pale and listless. During the ninth day of life it regurgitated its food. At this time the respirations were labored and shallow and reached the rate of 160 per minute. The child became rapidly more cyanotic and died late on the ninth day. No cardiac murmurs were described, but roentgenographic examination showed the heart to be markedly enlarged.

Description of Heart.—The dimensions of the abnormal heart are as follows:

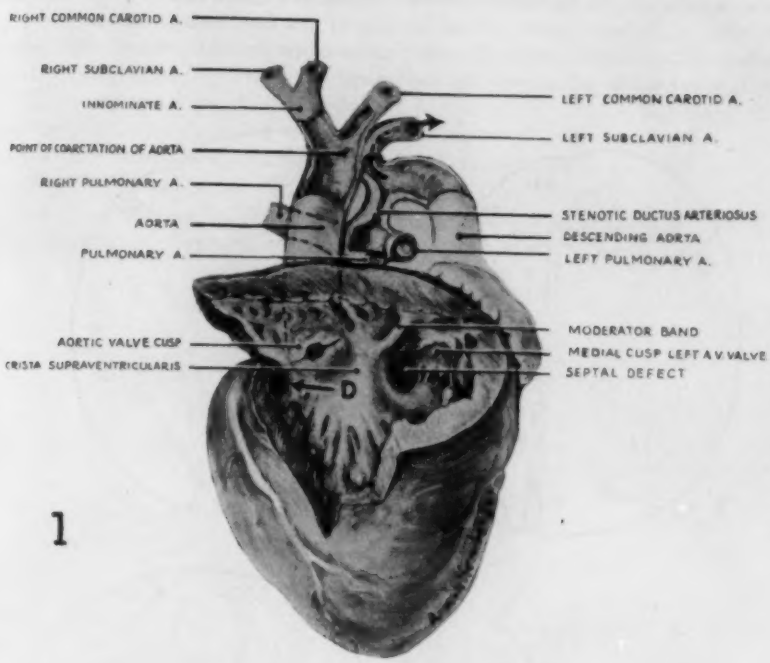
1. Length (apex to roof of right auricle)..... 6.0 cm.
2. Length of ventricular region (apex to base between the pulmonary artery and aorta) ..... 4.7 cm.
3. Greatest width (perpendicular to the axis in the frontal plane). 4.5 cm.
4. Depth (greatest anteroposterior diameter)..... 3.7 cm.
5. Thickness of the wall of the left-sided ventricle (at the middle of the chamber) ..... 4.0 mm.
6. Thickness of the wall of the right-sided ventricle..... 9.0 mm.

7. Circumference of aortic orifice (at the level of the commissures) ..... 1.9 cm.
8. Circumference of the pulmonic orifice..... 2.9 cm.
9. Circumference of the left atrioventricular orifice..... 2.5 cm.
10. Circumference of the right atrioventricular orifice..... 4.2 cm.

As viewed anteriorly, the anterior coronary sulcus is displaced far to the left. This indicates a disparity in the size of the ventricles, which is confirmed as soon as the chambers are opened. The vessel that gives rise to the arteries of the head takes origin anteriorly and throughout its course remains anterior to the pulmonary artery. Both the aorta and the pulmonary artery pass directly upward and do not twist one about the other (fig. 1).

The right atrium is much larger than the left, and its auricular appendage passes in front of the base of the pulmonary artery and presents on the anterior surface of the heart. The left auricle cannot be seen from the anterior aspect. The right auricle is capacious. It receives the superior and inferior venae cavae. Only vestigial valves remain, together with a small network of Chiari. The coronary sinus, which enters in its usual position, is guarded by a prominent thebesian valve. The right atrioventricular valve has three cusps, one of which is very small, and there are three papillary muscles. There is an anterior cusp, a left posterior or septal cusp, and a small right cusp. The right ventricle greatly exceeds the left in capacity. The apical region of the heart is composed entirely of the wall of this chamber. Above the muscular part of the interventricular septum there is an oval defect 7 by 11 mm. in diameter. Anterior to the defect there is a thick muscular ridge, to be described later. One leaflet of the left atrioventricular valve inserts by means of chordae tendineae into the inferior and posterior margins of the defect (figs. 1 and 2). Between the medial cusps of the right and left atrioventricular valves there arises along the posterior wall a column of muscle which is interpreted as a rudiment of the true posterior septum (see comment). Taking origin above the defect, and therefore straddling both ventricles, is a large pulmonary artery. The orifice of this is posterior to the posteromedial cusp of the right atrioventricular valve. It has three well defined cusps, as indicated in figure 6.

The left auricle receives the pulmonary veins in the usual manner. This auricle is brought into communication with the left-sided ventricle by a very narrow ring approximately 1 cm. in diameter, which is guarded by three cusps. One of these, the anteromedial, hangs like a curtain over the upper part of the interventricular septal defect, as has already been stated (figs. 1 and 2). There are two other well defined cusps, an anterolateral and a posterior. The left-sided ventricle is small and lies in a left lateral position. It is partially subdivided by an almost transverse muscular ridge, 7 mm. thick and 13 mm. wide, that arches over the upper part of the ventricle. Medially this ridge fuses with the septum and forms the anterior boundary of the interventricular septal defect. Anterior to this transverse ridge there is a deep crevice situated between it and the anterior wall of the heart, which medially is delimited by an intact muscular wall separating the two ventricles. Laterally the muscular ridge fuses with the wall of the left ventricle. There is, however, a space left between the free lower margin of this ridge and the floor of the left ventricle, through which the two portions of the left ventricle communicate. The aorta arises anteriorly, just behind the crevice previously mentioned (fig. 1). The transverse ridge of muscle thus appears to insert between the aorta and the pulmonary artery. It is interpreted as the crista supraventricularis. A



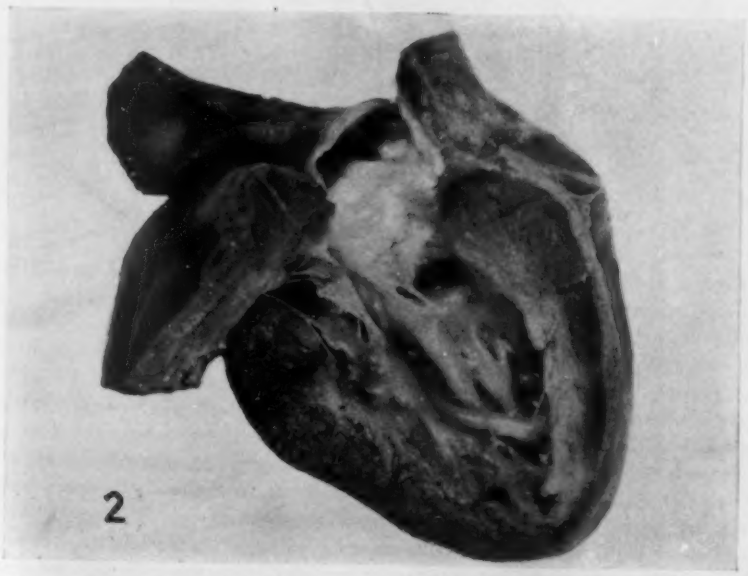
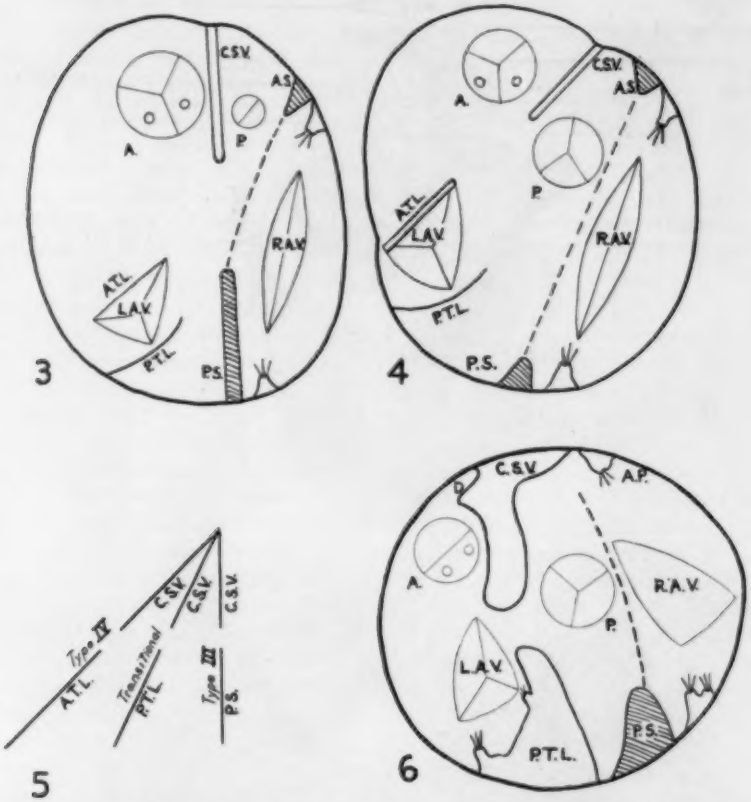


Fig. 1.—View of heart from the left side. Note relations of the great vessels. *D* indicates a depression in the anterior portion of the septum.

Fig. 2.—Photograph of right-sided ventricle. A vertical bar of muscle, interpreted to represent a rudiment of a true posterior septum, descends from the region between the medial cusps of the right and left atrioventricular valves.

bar of muscle 1.5 mm. in diameter corresponding to a moderator band extends from the crista to the lateral wall. From the base of this band medially there projects a papillary muscle which sends chordae tendineae to the anterolateral and anteromedial cusps of the left atrioventricular valve.



Figs. 3, 4, 5 and 6.—Figures 3 and 4 show the mirror images of types III and IV, respectively, of Spitzer,[1] diagramed according to the method of this writer. Figure 6 shows a similar diagram of the anomalies in case 1. Figure 5 shows how the plane of the crista supraventricularis in rotating from its position in type III (in line with the true posterior septum) to that in type IV (in line with the anterior tricuspid ledge) comes to be in line with the posterior tricuspid ledge, as in case 1.

In each case the theoretic position of the true septum between the right and the left ventricle is indicated by the broken line. In figure 6 the exact position of the vestige of the true anterior septum is not known.

Key: *A* is the aorta; *P*, the pulmonary artery; *L.A.V.*, the left atrioventricular orifice; *R.A.V.*, the right atrioventricular orifice; *A.S.*, the true anterior portion of the septum (or vestige); *P.S.*, the true posterior portion of the septum (or vestige); *C.S.V.*, the crista supraventricularis; *A.T.L.*, the anterior tricuspid ledge; *P.T.L.*, the posterior tricuspid ledge; *A.P.*, the anterior papillary muscle; *D*, the depression in the anterior portion of septum.

Histologic sections of the ventricles show no evidence of scarring or of an acute tissue response.

The aorta is guarded by two semilunar cusps. Its position at the point of origin is indicated in figure 1. There are tiny additional folds in the sinuses of Valsalva which produce ridges but do not completely subdivide each of these two cusps. The posteromedial sinus of Valsalva contains the orifices of both the major coronary vessels. One passes to the right and one to the left, as shown in the coronary artery diagram (fig. 7).

The Great Vessels.—The aorta at first is directed toward the right, but then bends upward and slightly to the left at about a right angle. The ascending portion

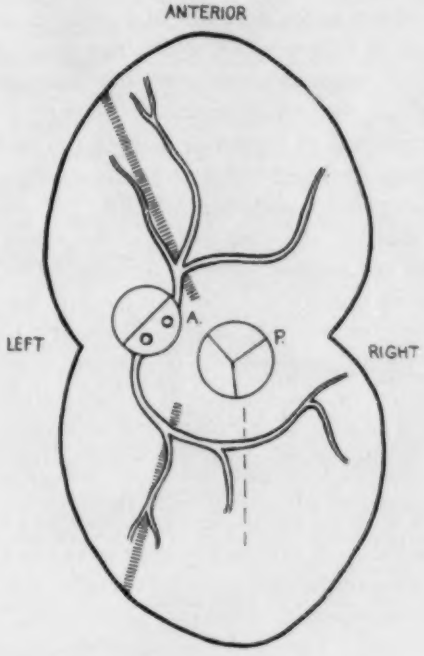


Fig. 7 (case 1).—Diagram of the positions of the coronary arteries and of the lines of attachment of the septums.

of the aorta gives rise on the right to the innominate artery, which after a course of about 1 cm. branches into the right subclavian and right common carotid arteries. Immediately adjacent to this orifice is that of the left common carotid artery. At this point the left subclavian artery bends from a point of origin in the descending aorta and becomes adherent to the group of vessels just described. A tiny dimple can be seen in the ascending portion of the aorta, where the left subclavian adheres. This is thought to represent a point of atresia which now separates the ascending portion of the aorta from the descending portion. The latter receives its blood by means of a thick-walled ductus arteriosus 6 mm. long which brings it into communication with the pulmonary artery at the point of bifurcation of the latter. The pulmonary artery is a huge vessel separated from the right ventricle by means of

the three large cusps previously described. The orifice of this vessel is just above the upper rim of the interventricular septal defect and posterior to the septal cusp of the right atrioventricular valve. The ductus arteriosus has a very tiny lumen and a thick resistant wall.

COMMENT ON CASE 1

Evidence of Bulboventricular Inversion.—The relations of the great vessels, aorta left anterior and pulmonary artery right posterior, suggests at once the existence of bulboventricular inversion (fig. 1). The almost parallel upward course of the vessels indicates complete failure of torsion.

Inspection of the left ventricle reveals a tricuspid atrioventricular valve. Further evidence is the presence of a pillar of muscle suggesting the moderator band on this side, on which the small anterior papillary muscle inserts. This also indicates that the anterior portion of the ventricular septum is the crista supraventricularis. The depression between this structure and the anterior wall of the heart (*D* in fig. 1) represents the forward directed curve of the ventricular continuation of the crista aorticopulmonalis as shown in Spitzer's [3] interpretation of the case reported by Wurm.[4] In that instance there was indeed a defect in the septum in this remarkable anterior position. The crista is situated between the pulmonary artery and the aorta. The latter therefore corresponds to the "right-chambered" aorta in Spitzer's interpretation.

Thus both the right-chambered aorta and the ventricle bearing the structures encountered on the right side in situs solitus are found on the left side here. The case is therefore one of inversion of the bulboventricular parts of the heart, since the auricles are typical in their relations.

The presence of three cusps in what corresponds to the mitral valve (in the present case the right atrioventricular valve) is not uncommon in the normal heart. One of the cusps is much smaller than the others, and there are, as would be expected in the case of the mitral valve, two large major papillary muscles. Furthermore, there are no structures in the right-sided ventricle of this case to indicate that it does not correspond to the usual mitral ventricle.

Evidence That the Posterior Half of the Septum Is the Posterior Tricuspid Ledge.—The papillary muscles that send cordae to the posterior and medial cusps of the left atrioventricular valve form a large part of the muscular bar that makes up the posterior half of the ventricular septum. Also the medial cusp of the valve bridges over the septal defect. This indicates the posterior part of the septum to be the posterior tricuspid ledge. The condition, then, is intermediate between types III and IV of Spitzer in inversion. In figures 3 and 4 are reproduced the mirror images of types III and IV. It is clear that

3. Spitzer, A.: Virchows Arch. f. path. Anat. **263**:142, 1927.
4. Wurm, H.: Virchows Arch. f. path. Anat. **263**:123, 1927.

as the crista aorticopulmonalis rotates from the position in the former to that in the latter, the plane of the posterior tricuspid ledge must be passed (figs. 3, 4 and 5). When the two come into line, the posterior tricuspid ledge hypertrophies, as do all septums situated between the two main currents of blood.

Confirmatory evidence is the presence of what is interpreted as a rudiment of the true posterior septum. This is a heavy vertical bar of muscle to the right of the posterior tricuspid ledge between the medial cusps of the right and left atrioventricular valves (figs. 2 and 6). A branch of the coronary artery courses down the posterior wall of the heart in line with this rudiment. The position of the anterior part of the septum is not certain from inspection of the heart but may be that of any one of a number of trabeculae on the anterior wall.

From this interpretation, as indicated in figure 6, the transposition is apparent rather than real, since only the mitral valve lies to the right (left in situs solitus) of the true interventricular septum.

Comparison with Other Examples of Bulboventricular Inversion.—The present instance tallies closely with the description of case 16 of Harris and Farber's [2] series. It is similar also to that of Roos [5] in the presence of coarctation of the aorta, and to that of Schmincke and Doerr,[6] in which the great vessels were anomalous in almost the same respects as those reported here. In the heart described by Wurm there was no defect between the anterior and posterior portions of the septum but a small defect lay between the anterior septum and the anterior wall of the heart. In the present case there was no defect here but a niche. Both probably represent, as suggested by Spitzer, the concavity of the C-shaped crista aorticopulmonalis, which in inversion and detorsion of types III and IV would face anteriorly. In Walmsley's [7] case the septum was entire. This results from obliteration of both the anterior and the posterior defect. Walmsley chose to ignore Spitzer's theory, but if this is applied to his case it becomes apparent that what was designated as crista supraventricularis is probably the bulboatrial ledge and that what was designated as the anterior portion of the interventricular septum is probably the crista. In that instance all of Spitzer's postulates are completely fulfilled.

CASE 2: DETORSION WITH RUDIMENT OF "RIGHT AORTA"

Dextroposition of aorta; persistent rudiment of "right aorta"; interventricular and interauricular septal defects; atresia of pulmonary artery;

5. Roos, A., cited by Abbott, M. E.: Atlas of Congenital Cardiac Disease, New York, American Heart Association, 1936, p. 58.

6. Schmincke, A., and Doerr, W.: Beitr. z. path. Anat. u. z. allg. Path. **103**: 416, 1939.

7. Walmsley, I.: J. Anat. **65**:528, 1931.

large defect in left aorticopulmonary septum and small defect in right aorticopulmonary septum, atrophy of crista supraventricularis; hypertrophy of right ventricle.

Clinical History.—A boy lived for only a few moments after delivery. Only a few gasping breaths were taken, and attempts at artificial respiration proved to be futile. The patient's mother was 28 years old and had had two children previously, aged 6 and 9 years, respectively. The latter was said to have some variety of heart disease.

The present pregnancy and labor were said to be uncomplicated, and the infant weighed 8 pounds (3,629 Gm.).

Description of Heart.—The dimensions of the anomalous heart are as follows:

1.	Length (apex to roof of right auricle)	5.5 cm.
2.	Length of ventricular region (apex to base between the pulmonary artery and aorta)	3.9 cm.
3.	Greatest width (perpendicular to axis in frontal plane)	4.5 cm.
4.	Depth (greatest anteroposterior diameter)	3.5 cm.
5.	Thickness of wall of left-sided ventricle (at middle of chamber)	5.0 mm.
6.	Thickness of wall of right-sided ventricle	8.0 mm.
7.	Circumference of aortic orifice (at level of commissures)	2.5 cm.
7a.	Circumference of "right aortic" orifice	0.9 cm.
8.	Circumference of pulmonic orifice	0.0 cm.
9.	Circumference of left atrioventricular orifice	3.3 cm.
10.	Circumference of right atrioventricular orifice	3.5 cm.

The heart has the outline of an obliquely placed rectangle. The apex appears directed toward the right and is made up of what appears to be the right ventricle. A sulcus containing coronary vessels, however, proceeds directly downward on the anterior surface. The right auricular appendage is visibile from the anterior aspect as it projects from behind the right ventricle. The small left auricular appendage is completely hidden from view as the heart is seen from the front.

The right auricle receives the inferior vena cava which enters by an orifice 7 mm. in diameter. The superior vena cava has an orifice only 3 mm. in diameter. The latter vessel is guarded anteriorly by a delicate endocardial network of Chiari. The coronary sinus enters at its usual position through a slitlike orifice with a small thebesian valve. An interauricular septal defect 5 mm. in diameter is seen to be partially covered on the right side by an anterior semilunar ridge and on the left side by a large posterior flap of endocardium. Three cusps guard the right atrioventricular orifice. There are an anterior and a posterior cusp, each 1.5 cm. at its insertion, and a right cusp that at its insertion measures only 0.5 cm. The medial wall of the right chamber has thick trabeculae carneae with deep interstices, but on the anterodextral wall the trabeculae appear flattened. Trabeculae are scarcely visible on the left side since they are so flattened and thin. The capacity of the right ventricle exceeds that of the left by about one third. Much the greater part of the right ventricle is situated anteriorly and to the left of the anterior cusp of the tricuspid valve. In the upper part of the interventricular septum, anteriorly to this cusp, there is an oval defect measuring 10 by 8 mm. A large aortic orifice, 9 mm. in diameter, overrides this defect. This orifice is

bounded by three well formed cusps as indicated in the diagram (fig. 9). To the right and anteriorly there is a much smaller orifice, measuring 4 mm. in diameter, which is outlined by ill defined endocardial folds, resembling poorly developed cusps. A probe passed through this orifice enters a pocket whose wall resembles that of the aorta. This sac terminates blindly against the left wall of the pulmonary artery except for a small defect medially near its origin, which produces a communication with what appears to be the pulmonary trunk. Directly anteriorly and to the left of the orifice of this sac there may be seen from the ventricular aspect a pit whose base is a thin membrane that separates the cavity of the ventricle from the pulmonary artery. The condition thus seems to be one of obliteration of the pulmonary artery at its origin and persistence of the "right aorta." The anterior papillary muscle inserts into a broad and short moderator band. As the latter approaches the septum it is lost, and there is no clearly

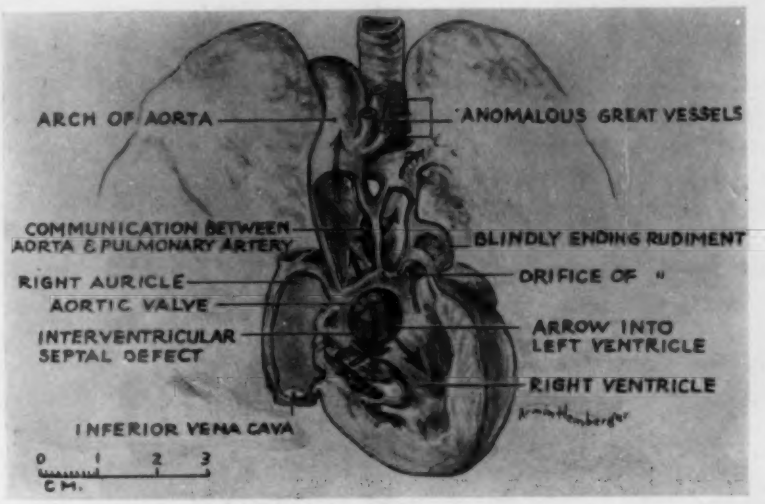
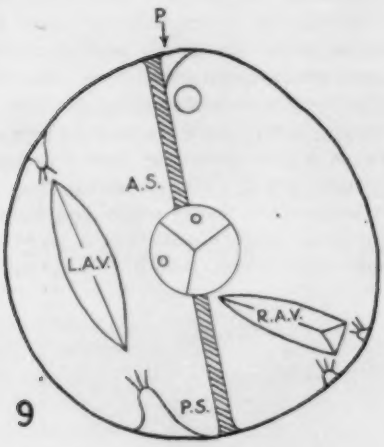


Fig. 8 (case 2).—Heart viewed anteriorly and from the right; aortic arch viewed anteriorly. The lower parts of the ascending positions of the great vessels are somewhat twisted. A part of the right ventricle is imagined as having been cut away.

defined muscular crista supraventricularis. The ridge of tissue between the obliterated pulmonary orifice and the "right aorta" is thought to represent the crista. Its position is indicated in the detailed diagram (fig. 9).

The left auricle receives two main pulmonary veins from the right lung and one from the left. The atrioventricular valve is bicuspid. The left ventricle in large part lies anteromedially to the anterior cusp. The interventricular septal defect has already been described. The aorta straddles the defect in such a manner that only one third of the circumference of the base overlies the left ventricle.

The Great Vessels.—The dome-shaped projection which has been called the "right aorta" above extends only 8 mm. beyond its rudimentary valve ring. The pulmonary artery takes origin anteriorly and to the left of the rudiment, but this then twists about to terminate on the left wall of the pulmonary trunk. The



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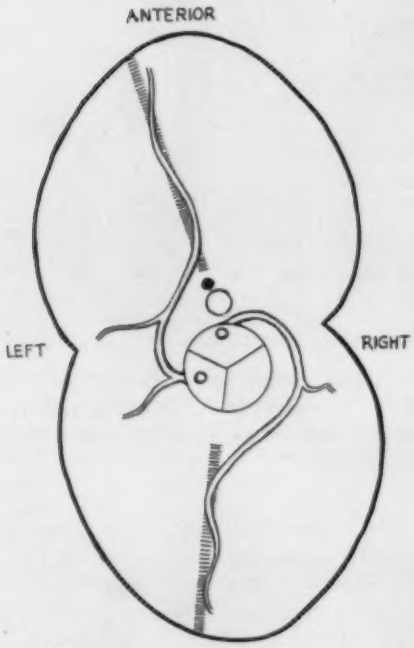

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Fig. 9 (case 2).—Diagram of the base of the heart. Key as in figures 3 to 6.

Fig. 10 (case 2).—Diagram of the coronary arteries.

latter terminates inferiorly in a thin membrane which separates it from the right ventricle. It therefore communicates with this chamber only in an indirect way, by means of the minute defect in the wall of the "right aortic rudiment" and by means of a very large defect in the left aorticopulmonary septum. This defect begins 3 mm. above the left aortic valve ring and is 8 mm. long and 6 mm. wide. The upper part of the pulmonary artery lies anteriorly and to the left of the "left aorta." Higher still the aorta begins to swing anteriorly, but after giving off great vessels it arches to the right over the right main bronchus and descends on the right side of the vertebral column. The distribution of the coronary vessels is shown in figure 10.

Microscopic sections of the walls of both ventricles show well preserved muscle and epicardium and endocardium. The wall of the "right aortic rudiment" has exactly the same fibro-elastic structure as the aorta and pulmonary artery themselves.

COMMENT ON CASE 2

In Spitzer's theory, the septum that theoretically exists between the right and the left aorta is merely stated to disappear as the left aorta becomes united with the right in detorsion. In the present instance a cross section 2 mm. above the base of the ventricle would show three lumens of three great vessels separated by septums as in the theoretic reptilian-like aorta of the ancestral form. The blind termination of the pulmonary artery on the base of the right ventricle, from which it is separated by a thin membrane, has already been described. The rudimentary structure that takes origin dextroposteriorly to this membrane may therefore represent the "right aorta," delimited from the left in this instance by a persistent interaortic septum. Between the pulmonary artery and this "right aorta" should, therefore, exist the aorticopulmonary septum and a downward continuation, the crista supraventricularis. The latter should indeed show hypertrophy in detorsion of types I and II. But with persistence of the right aortic rudiment and atresia of the pulmonary artery the crista is atrophic. The trabecula septomarginalis, however, continues downward in a large moderator band, on which the anterior papillary muscle of the tricuspid valve inserts in the usual manner. It can be imagined that with the counterclockwise detorsion the upper end of the "right aortic rudiment" has been drawn farther to the left than the proximal. The only outlet of the rudiment is the small communication with the pulmonary artery that has been described.

There is no evidence of stenosis of the pulmonary conus at the origin of the bulbus in the sense of Keith.[8] There is a definite infundibulum or outflow portion of the right ventricle, represented by the ample part of that chamber beyond the moderator band. What is considered the "right aortic rudiment" has all the characters of an independent structure, as

8. Keith, A.: Lancet **2**:433, 1909. Eakin, W. W., and Abbott, M. E.: Am. J. M. Sc. **186**:860, 1933.

evidenced by its tapering termination high on the left wall of the pulmonary artery. It is furthermore inconceivable that the rudiment represents merely the proximal portion of the pulmonary artery recurved on itself, since the latter has a separate funnel-shaped termination directly on the roof of the right ventricle. The wall of the rudiment, moreover, contains no cardiac muscle and has at all points a structure resembling that of the aorta and pulmonary artery at their bases.

SUMMARY

In this study Spitzer's theory has received confirmation (1) in the analysis of a very complex anomaly, bulboventricular inversion with transposition of the great vessels, and (2) in suggestive direct evidence of the existence of a homologue of the reptilian right aorta, which is recorded for the first time as observed in an anomalous human heart.

Drs. Sidney Farber and Henry W. Edmonds gave valuable advice as to the discussion of the material presented here.

EXPERIMENTAL LESIONS PRODUCED BY CEREBROSIDES

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In Gaucher's disease there is an abnormal storage of a large quantity of cerebrosides within the cells of the reticuloendothelial system. Thannhauser and Magendantz [1] postulated that the condition is caused by a disturbance of the intracellular metabolism of cerebrosides. Pick [2] and most other investigators of this problem, however, have assumed that the disease is caused by a general disturbance of the metabolism of cerebrosides and that the lipid thus produced in excess is stored in the reticuloendothelial system.

Few attempts have been made to produce the disease in animals by the administration of cerebrosides. Beumer and Fasold [3] injected intravenously small quantities of cerebrosides of unspecified purity for a few days. They stated that there was an increase of cerebrosides in the liver but that no other sites were examined. Pasternak and Page [4] gave intraperitoneal, intramuscular and subcutaneous injections of cerebrosides, but apparently they studied only the local lesions produced. Kimmelstiel and Laas [5] injected considerable quantities of cerebrosides over a period of several weeks. Histologically, they noted deposition of the lipid in the liver, spleen and lymph nodes. Chemical analyses of the blood or tissues were not made.

In view of the dearth of experiments related to the production of Gaucher's disease in animals it was considered desirable to study this problem further. A highly purified cerebroside preparation [6] was administered orally and intraperitoneally to rabbits. During the experi-

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This investigation was aided by a grant from the University Research Fund.

From the John Jay Borland Fellowship for Clinical Research of the Henry Baird Favill Laboratory of St. Luke's Hospital, Chicago, and the Department of Pathology of the University of Alabama Medical School.

1. Thannhauser, S. J., and Magendantz, H.: Ann. Int. Med. **11**:1662, 1938.

2. Pick, L.: Klin. Wchnschr. **4**:1793, 1925.

3. Beumer, H., and Fasold, H.: Ztschr. f. d. ges. exper. Med. **90**:661, 1933.

4. Pasternak, L., and Page, I.: Biochem. Ztschr. **252**:255, 1932.

5. Kimmelstiel, P., and Laas, E.: Beitr. z. path. Anat. u. z. allg. Path. **93**:417, 1934.

6. The crude cerebrosides were obtanied through the aid of Dr. Frederic Fenger, director of clinical and therapeutic research of Armour and Company, Chicago.

ment the values of the blood lipids, the blood counts, the temperatures and the weights of the animals were recorded at regular intervals. Finally, when the animals were killed, the tissues were examined grossly, histologically and chemically.

MATERIALS AND METHODS

Preparation of the Cerebrosides.—The cerebrosides were obtained in a crude form by extracting fresh calf brain with boiling alcohol. The extract was filtered while hot, and when it had cooled a mixture of lipids, including cerebrosides, precipitated. The precipitate was repeatedly extracted with absolute alcohol, chloroform, acetone and purified petroleum benzine U. S. P. (petroleum ether). One hundred and thirty pounds of fresh brain yielded 74 Gm. of nearly pure cerebrosides. The product contained 0.3 per cent sphyngomyelin but no lecithin, cephalin, cholesterol or other lipids.

Administration of the Cerebrosides.—Rabbit 2 received 1 Gm. of the lipid by mouth daily for ninety-nine days. Three other rabbits received intraperitoneal injections of a 2.5 per cent sterile emulsion of cerebrosides in distilled water. The emulsion was prepared by dissolving the lipid in hot absolute alcohol; the latter solution was poured slowly into boiling distilled water, and heating was continued until the alcohol had evaporated. Rabbits 3 and 5 received 20 cc. of this emulsion intraperitoneally every other day for eighty-nine and twenty-four days, respectively. Rabbit 4 received the same amount every other day for fifty-one days and then daily for twenty-seven days. All rabbits, including control rabbit 1, were fed oats and occasional fresh carrots and lettuce.

At intervals of two to three weeks during the administration of the lipids the following determinations were recorded: red and white blood cell and differential counts; hemoglobin content of the blood; weight; rectal temperature; results of a quantitative analysis of the plasma lipids.

All animals were killed with ether, and autopsies were performed within twenty-four hours after the last administration of cerebrosides. Tissues were fixed in Zenker's solution and in solution of formaldehyde U. S. P. (1:10). Those fixed in the formaldehyde solution were sectioned by the freezing method, stained with scarlet red and counterstained with hematoxylin. Tissues fixed in Zenker's solution were embedded in paraffin, cut and stained with hematoxylin and eosin, Masson's trichome stain,[7] hematoxylin and eosin, and Mallory's aniline blue stain.

Chemical Analysis of Tissues.—The following tissues were examined chemically for their lipid constituents immediately after the autopsy: lung, liver, kidney, myocardium, cerebrum, lymph node, retroperitoneal fat, adrenal gland, bone marrow from the femur and spleen. About 1 Gm. portions of fresh tissue were weighed, ground finely with lipid-free purified sand in a mortar and then transferred quantitatively into an extraction flask. The material was extracted with boiling redistilled 95 per cent alcohol and ether (3:1) for two hours. The alcohol-ether extract and the ether washings were filtered through a fat-free paper filter into a 100 cc. volumetric flask and made up to 100 cc. volume with ether.

The following lipid determinations were made:

1. Total Lipids: A 30 cc. portion of the extract was quantitatively poured into a porcelain crucible, the solvent evaporated at room temperature and the crucible dried to constant weight in a desiccator.

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7. Masson, P.: J. Tech. Methods **12**:75, 1929.

2. Phosphatides: A 40 cc. aliquot of the alcohol-ether extract was pipetted into a beaker and evaporated to dryness in the incubator (37 C.) overnight. The lipids were redissolved by repeated extractions with warm purified petroleum benzine. The extract was poured into a 50 cc. centrifuge tube and evaporated to about 1 cc. by placing the tube in warm water. Then the ether-soluble phosphatides (lecithin, cephalin) were separated from the ether-insoluble lipid (sphyngomyelin), according to Boyd,[8] by adding 7 cc. of acetone and 20 cc. of freshly redistilled, peroxide-free ether saturated with distilled water. The amount of phosphatide was ascertained by determining the phosphorus in each fraction by the Youngburg method.[9]

3. Total Cholesterol: A 20 cc. portion of the alcohol-ether extract was placed in a beaker and saponified by adding 10 drops of a saturated aqueous solution of potassium hydroxide and gently boiling on a water bath for one hour. The saponified lipids were extracted several times with purified petroleum benzine, and the total cholesterol was determined by the Boyd method.[10]

4. Cerebrosides: An attempt was made to determine the amount of cerebrosides indirectly by finding the amount of galactoside by Kirk's method.[11] The results, however, were not dependable, probably because of the presence of varying amounts of unknown reducing substances in the tissues. The attempt, therefore, was abandoned. Instead, the percentage of residual lipid obtained by subtracting the combined weights of the phosphatides and total cholesterol from the total lipid gave a rough index of the combined amount of fatty acids, neutral fats and cerebrosides.

GROSS EXAMINATION

Abdominal Cavity.—In rabbits 3, 4 and 5 there were edematous and vascular granulation tissues between the abdominal wall and several loops of bowel in the region of the injections. In the granulation tissues were many gray encapsulated nodules, ranging to 1 cm. in diameter. These consisted of a soft white cheeselike material. There were numerous similar nodules, a few millimeters in diameter, in the parietal and visceral peritoneum, the mesentery, the capsule of the liver and spleen and the serosa of the bowel. All of these changes were most marked in rabbit 4 and least in rabbit 5. No changes were noted in rabbits 1 and 2.

Spleen.—The spleens of the rabbits that received large amounts of cerebrosides were moderately enlarged. The weights of the spleens of rabbits 1, 2, 3, 4 and 5 expressed in percentages of body weight were 0.2, 0.6, 0.6, 0.9 and 0.1, respectively. The splenic tissue of rabbits 2 and 4 was firm, and the malpighian bodies were indistinct because of irregular streaks of gray tissue in the red pulp. The firmness and the gray tissue were not as pronounced in rabbit 3. In rabbits 1 and 5 there were no gross alterations.

Liver.—The liver from rabbit 4 was firm and light gray, and the lobular markings were obscure. Also the liver from rabbit 2 had similar but not such marked changes. No appreciable changes were noted in the other livers.

Lymph Nodes.—The lymph nodes in all animals except rabbit 1 were enlarged. The most general enlargement, including mesenteric, perigastric and tracheobronchial lymph nodes, occurred in rabbit 4. The tracheobronchial lymph nodes in the

8. Boyd, E.: Am. J. Clin. Path. (Tech. Supp.) **2**:77, 1938.

9. Youngburg, G. E., and Youngburg, M. V.: J. Lab. & Clin. Med. **16**:158, 1930.

10. Boyd, E. M.: Am. J. Clin. Path. (Tech. Supp.) **1**:83, 1938.

11. Kirk, E.: J. Biol. Chem. **123**:613, 1938.

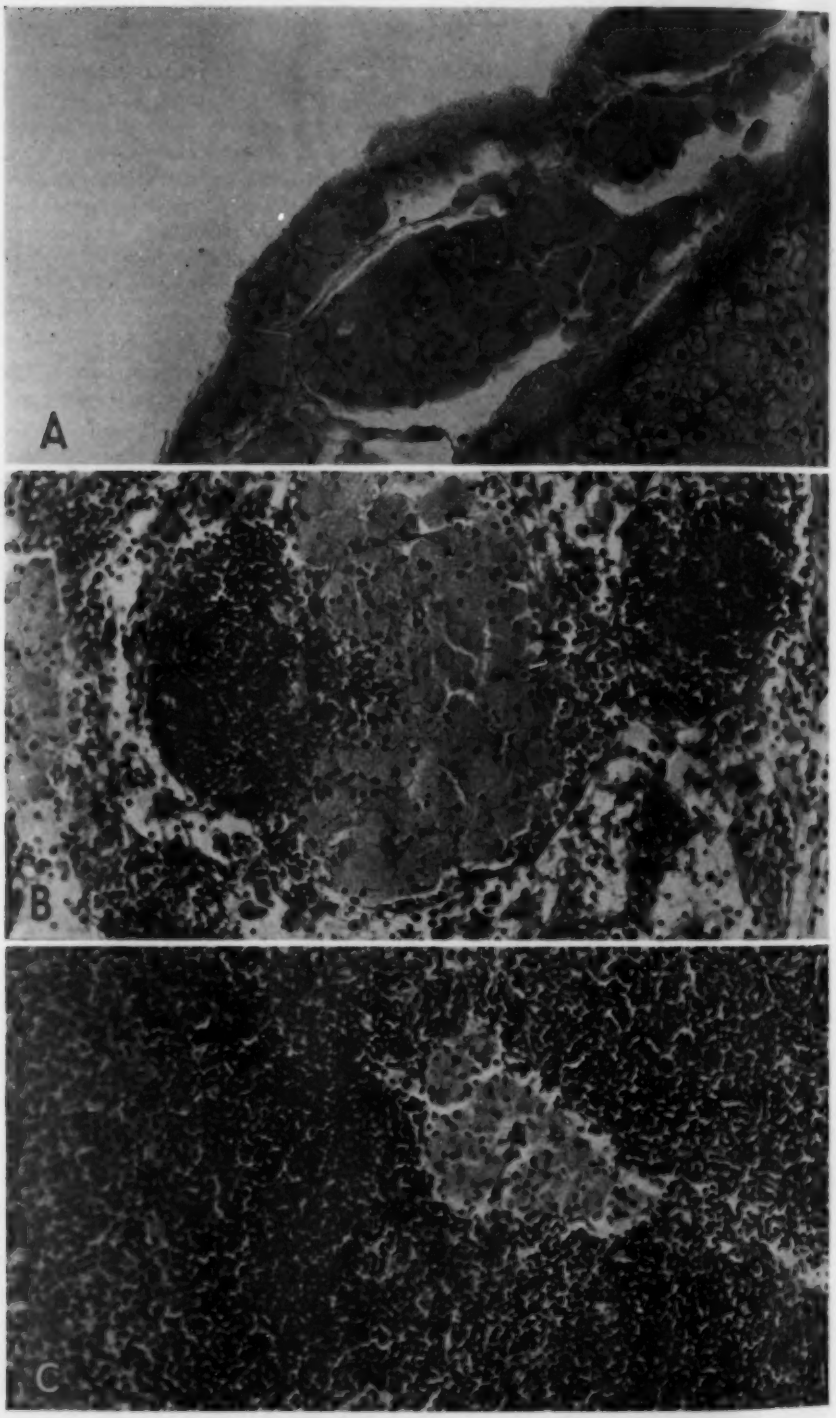

Figure 1

(See legend on opposite page)

other rabbits were not appreciably enlarged. The nodes ranged to 1.5 cm. in diameter in the root of the mesentery and less elsewhere. The lymphoid tissue was uniformly light gray and slightly edematous.

Other Tissues.—There were no noteworthy gross changes in the following tissues of any rabbit: lung, brain, kidney, adrenal gland, bone marrow of the femur, ovaries, fallopian tubes, gastrointestinal tract, pancreas and heart.

MICROSCOPIC EXAMINATION

Gray Nodules in Abdominal Viscera.—The gray nodules in the abdominal viscera consisted of masses of large round to polygon-shaped cells interspersed in a sparse granulation tissue (figure 1 *A*). The cells had from one to several small vesicular nuclei each and an abundance of coarsely and finely vacuolated cytoplasm that stained pale blue with Mallory's aniline blue. In tissues stained for fat with scarlet red, the vacuolated cytoplasm became pale yellow-orange. There was only a slight fibroblastic tissue response in the small nodules, whereas in the large masses the centrally located cells had disintegrated, and around this necrotic region was considerable vascular granulation tissue.

Lymph Nodes.—The mesenteric, perigastric and peritracheal lymph nodes of rabbit 4 revealed hyperplasia of the germinal centers and particularly of the reticuloendothelial cells. Arranged singly and in clusters, there were cells varying in size from an ordinary phagocytic cell to the large foam cells observed in the nodules described (fig. 1 *B*). These were in the germinal follicles and pulp cords, and also a few were within the sinuses. There was an occasional giant cell with a foamy cytoplasm. The cells lining the sinuses were not appreciably altered. The aforementioned changes were less pronounced in rabbit 3 and least in rabbit 5.

In rabbit 2 there was also hyperplasia of the reticulum cells, and within the sinuses was a considerable number of large mononuclear cells with the usual-appearing cytoplasm. No foam or giant cells were observed, and the cytoplasm of the proliferated reticulum cells did not stain with Mallory's aniline blue. The lining cells of the sinuses were not conspicuous.

Spleen.—In the spleens of rabbits 3 and 4 and to a slight extent in that of rabbit 5 there was hyperplasia of the malpighian bodies and particularly of the reticulum cells of the pulp cords. Foam cells occurred singly as well as in clusters in the germinal centers and in the cords (fig. 1 *C*). Occasional giant cells were noted. All gradations from the typical reticulum cell to the foam cell were present, and all were identical in their staining qualities with similar cells observed in the lymph nodes. Occasional foam and giant cells were within sinuses. The latter had an unchanged lining and were usually empty and compressed by the hyperplastic pulp cords. No foam cells were observed in the spleen of rabbit 5.

In the spleen of rabbit 2 there was also marked hyperplasia of the reticulum cells of the pulp cords and particularly at the periphery of the malpighian bodies. There were, however, no foam or giant cells and no cells stained with Mallory's

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EXPLANATION OF FIGURE 1

A, nodule in the capsule of the liver of rabbit 4; × 198. Note the large vacuolated mononuclear and multinucleated cells. *B*, abdominal lymph node from rabbit 4; × 198. Note the replacement of lymphoid tissue by large foam cells. *C*, photomicrograph of spleen from rabbit 4; × 198. There are clusters of foam cells in the malpighian bodies.

aniline blue. The noteworthy change was the enlargement of the cells lining the sinuses. They had an abundant clear, glassy and slightly granular appearance and did not stain with Mallory's aniline blue or with scarlet red.

Liver.—Rabbits 3 and 4 had the only changes observed in the liver. The liver of rabbit 4 showed numerous markedly enlarged Kupffer cells. They had an abundant finely vacuolated cytoplasm with staining qualities simliar to those of the vacuolated reticuloendothelial cells described in other tissues. There were a few foam cells within lymphatic channels in the portal regions. Like changes were present but less conspicuous in the liver of rabbit 3 and not present in the livers of rabbits 1, 2 and 5.

Retroperitoneal Fat Tissues.—Near the peritoneal margin of the adipose tissue occurred foam cells, singly and in clusters, many of which seemed to be within dilated lymph channels. A few were between fat cells.

Sections of myocardium, lung, kidney, cerebrum, ileum, colon, femoral bone marrow, fallopian tube, adrenal gland, ovary and pancreas had no noteworthy alterations.

Variations in the Lipid Content of the Blood Plasma of Rabbit 3

	Before Injection	35 Days †	57 Days	71 Days	85 Days	80 Days
Total lipid *	0.800	1.740	1.195	1.183	1.124	1.200
Lecithin and cephalin *	0.280	0.290	0.200	0.168	0.160	0.175
Total cholesterol *	0.120	0.156	0.140	0.142	0.098	0.095
Syringomyelin *	0.197	0.216	0.310	0.240	0.140	0.150

* The values are expressed as grams per hundred cubic centimeters of plasma.

† Each number of days represents the duration of the experiment after the injection of cerebrosides was begun.

RESULTS OF CHEMICAL ANALYSIS

Blood Plasma.—In the table are recorded the changes occurring in the concentrations of the several plasma lipids of rabbit 3. The fluctuations in the lipid values of rabbits 4 and 5 were generally about the same. The total lipid values were high, and this may be due partly to the fact that the weighed alcohol-ether extract contained nonlipid impurities and also because the dried lipid probably still retained moisture owing to the presence of the hygroscopic phospholipids.

The total fat content rose rapidly during the first month, then gradually dropped to a lower but considerably higher level than the original. The sphyngomyelin level in rabbits 4 and 3 rose considerably after about four weeks but dropped slightly in rabbit 5. After about three months the level dropped to approximately the original value. The lecithin and cephalin contents tended to decrease after four weeks. The cholesterol level remained nearly constant in all animals.

The only difference in the variation of the plasma lipid levels of the rabbit receiving cerebrosides orally from the rabbits getting the lipid intraperitoneally was the gradual rise in the lecithin-cephalin fraction to about double the original amount over a period of two months. In the ensuing month it gradually dropped to the original level. In control rabbit 1, observed for two months, there were no appreciable changes in the lipid content of the plasma.

Tissues.—It has been noted in the reports of investigators that the values recorded for a given lipid of any organ vary widely. This is presumably due partly to the use of different quantitative procedures and to the high percentage

of error in determining lipids in a small amount of tissue. Therefore, unless the values for lipids of the tissues differed markedly from those of the control animal, they were disregarded.

The organs with a definite increase in some lipid content were the liver, spleen and lymph nodes of rabbits 2, 3 and 4. These changes are recorded graphically in figure 2.

The total cholesterol and total phosphatide fractions did not vary appreciably. However, the lipid fraction remaining after subtracting the phosphatide and cholesterol contents from the total fat content was significantly increased in these three tissues, most marked in the lymph nodes and least in the liver. Of course, it can only be conjectured that this marked increase was caused chiefly by the cerebrosides, but this seems probable when the facts here are correlated with the findings in the histologic preparations of these tissues.

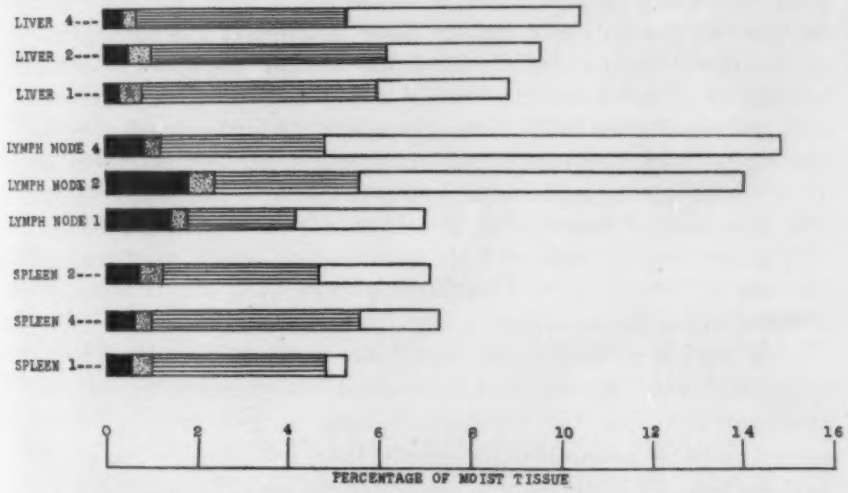

Fig. 2.—Diagram showing results of chemical analyses of tissues. The black spaces represent the percentage of sphyngomyelin. The dotted spaces represent the percentage of cholesterol. The horizontally ruled spaces represent the percentage of the lecithin-cephalin fraction. The clear spaces represent the percentage of neutral fat plus cerebrosides.

MISCELLANEOUS OBSERVATIONS

The red blood cell count and the hemoglobin content did not vary significantly for any rabbit during the experiment. In the rabbits receiving intraperitoneal injections there was moderate leukocytosis. This was interpreted as being caused by the local inflammation at the site of the injections. In these rabbits the temperature also rose slightly at intervals. The differential blood counts revealed no noteworthy changes in the morphologic characters or relative proportions of the cells. All rabbits gained slightly in weight.

COMMENT

The intraperitoneally injected aqueous emulsion of cerebrosides was apparently phagocytosed rapidly and easily by the large mononuclear

cells. Since this lipid is inert chemically and was present in a fine emulsion, it was taken up by the phagocytes with a minimal amount of inflammatory reaction. A moderate amount of granulation tissue formed where the center of large nodules composed of lipid-laden phagocytes had undergone necrosis. The mononuclear cells carried the lipid into the nearby lymphatic channels and nodes and then to widely scattered channels and nodes. It is uncertain whether the cerebrosides were metabolized within the phagocytes, but from a study of the staining characteristics of the engulfed material it seems probable that this was mainly stored.

Histologic examination of the organs from the several rabbits receiving varying amounts of cerebrosides seemed to indicate that for some time the lymphatic system absorbed and stored the lipid. Then the material found its way into the blood stream and was subsequently phagocytosed by the reticuloendothelial system elsewhere. Marked hyperplasia of the reticulum cells of the spleen ensued. Large foam cells and a few giant cells formed, in which the lipid content gave the staining qualities displayed by the cerebrosides engulfed by the mononuclear cells in the lymphatic channels. In rabbit 4, which received the largest amount of cerebrosides, a considerable number of the Kupffer cells of the liver, as well as of the mononuclear cells in the lymphatic channels of this organ, were markedly swollen by a similar deposition of lipids within the cytoplasm.

The marked increase in the total lipid content of the lymph nodes, spleen and liver following intraperitoneal injections of cerebrosides correlates well with the histologic changes of these tissues. There seemed to be no noteworthy increase in the phosphatide or the cholesterol fraction. Only the fraction containing the undetermined lipids was high. Since the fat stain revealed no neutral fats, the rise seems indicative of an increase in the amount of cerebrosides.

The changes in the tissues were essentially the same as those described by Kimmelstiel and Laas, who injected emulsions of cerebrosides intravenously. It certainly seems credible that if the injections could have been carried out over a longer period, or if larger quantities of cerebrosides had been injected, the alterations in the tissues would have approached those observed in Gaucher's disease.

In the past, many investigators have attempted to differentiate between Niemann-Pick disease, a disturbance of the metabolism of phosphatides, and Gaucher's disease by the type and location of the so-called foam cells. According to this view, in Niemann-Pick disease the phosphatides are deposited in the phagocytic cells of the splenic pulp cords, whereas in Gaucher's disease the cerebrosides are phagocytosed by the cells lining the sinuses. In this investigation, in which known cerebrosides were administered, the outstanding change in the

tissues examined was a proliferation of the reticulum cells of the spleen and lymph nodes, and nearly all cells containing the lipid were in the pulp cords or germinal centers.

The alterations of tissues in the rabbit receiving cerebrosides orally were different from those in rabbits that received the lipid intraperitoneally. This was expected because, according to Bloor,[12] cerebrosides are not absorbed from the gastrointestinal tract in an unchanged condition. That some form of lipid was being absorbed was suggested by the marked increase in total lipid and a gradual increase in the lecithin-cephalin fraction of the blood plasma. This was further suggested by the marked increase in the lipid content of the spleen, lymph nodes and liver, in addition to the hyperplasia of the spleen, the marked enlargement and some proliferation of the lining cells of the sinuses and the marked hyperplasia of the reticulum cells of the splenic cords. The nature of the stored lipid is not known. The enlarged cells had a clear glassy and slightly granular cytoplasm that did not stain with Mallory's aniline blue or with scarlet red. It is suggested that the lipid might be one of the split products of cerebrosides, such as sphyngosin.

In this rabbit there was a gradual rise to about double the original level of the lecithin-cephalin fraction of the blood plasma, and after about two months this fraction, as well as the total lipid, gradually decreased. It has been noted by other investigators that in lipemia occurring, for instance, in diabetes mellitus, there often is a rise in all the lipids of the blood. Weinhouse and Hirsch [13] produced cholesteremia in rabbits and noted a marked rise in other lipid components of the blood. A similar rise in various lipid fractions of the blood, therefore, seems probable if the cerebroside component is increased.

SUMMARY

A study was made of the effects of administering pure cerebrosides intraperitoneally and orally to rabbits. The total lipid and sphyngomyelin levels of the blood plasma rose in the rabbits receiving cerebrosides intraperitoneally. The lecithin-cephalin and cholesterol fractions did not vary appreciably. When the cerebrosides were ingested, the lecithin-cephalin fraction of the blood was increased, as well as the total lipid and the sphyngomyelin fraction. The lipid fraction containing the cerebrosides was increased in the liver, spleen and lymph nodes.

The morphologic changes noted in the tissues of rabbits receiving the lipid intraperitoneally were like but less extensive than those found in Gaucher's disease in man.

12. Bloor, W. R.: Physiol. Rev. **19**:557, 1939.
13. Weinhouse, S., and Hirsch, E. F.: Arch. Path. **30**:856, 1940.

SICKLE CELL DISEASE

TWO CASES, ONE PRESENTING FAT EMBOLISM AS A FATAL COMPLICATION

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Since Herrick,[1] in 1910, described sickle-shaped red cells in the blood of an anemic Negro man of 20 years, many similar cases have been reported and the true nature of this condition has been recognized. It owes its presence to a constitutional tendency of the bone marrow to produce elongated semilunar erythrocytes, which are treated as abnormal cells by the body defenses and are destroyed.[2] In many instances this destruction is sufficiently extensive to be the cause of hemolytic anemia. In other cases the elongated shapes of the erythrocytes make their passage through the capillaries difficult, thus producing a stagnation of the cells in the finer blood vessels of the different organs. Thromboses and endarteritis of different blood vessels, necroses of organs and fibrotic processes in certain viscera have been considered the direct and indirect results of such stagnation.[3] A large number of complications have been described in cases of this condition, among which must be mentioned fat embolism.

The blood of different patients shows wide variation in the proportions of sickle cell erythrocytes. When only a few sickle cells are present and the individual does not display any signs or symptoms referable to this abnormality, the condition is termed sicklemia. In other cases many elongated semilunar cells are present in the circulation and produce characteristic signs of disease; when this occurs, the condition is known as sickle cell anemia or, more appropriately, sickle cell disease. The clinical picture is a variable one, and the course may be rapid, ending in death in the first decade of life, or it may be prolonged more or less chronically into adult life. The severe type attacks its victims in infancy and displays periods of active disease alternating with periods of quiescence. During the stages of exacerbation the patients may com-

From the Office of the Chief Medical Examiner of the City of New York.

1. Herrick, J. B.: Arch. Int. Med. **6**:517, 1910.
2. Steinberg, B.: Arch. Path. **9**:876, 1930.
3. Bauer, J.: Arch. Surg. **41**:1344, 1940.

plain of pains in the abdomen with nausea and vomiting, migratory pains in the joints and signs of cardiac distress. Hemolytic anemia may occur, with yellowish green discoloration of the scleras. If the disease is sufficiently prolonged, persistent ulcers of a nonvaricose type make their appearance on the leg just above the ankle. Death occurs early, either as the result of the severe anemia or from some intercurrent infection.[4]

The pathologic changes found in the early stages are referable to the organic congestion with elongated sickle cells and the lysis of these cells by the reticuloendothelial system. The spleen is the organ chiefly concerned in this process and, as a consequence, shows the most striking pathologic changes. During acute exacerbations it is enlarged and dark purplish red, with a thin capsule and a smooth external surface. Haden and Evans [5] reported that spleens removed from children with sickle cell disease, either at necropsy or at operation, varied in weight from 112 to 665 Gm. On cut section the trabeculae are inconspicuous and the malpighian follicles are small and widely separated in the midst of swollen, dark red velvety parenchyma. This appearance is due to perivascular hemorrhages from the terminal portions of the splenic arterioles and the dilatation of the capillaries at the outer rim of the malpighian bodies, with the formation of pools of blood.[6] Microscopically, the splenic reticulum is stuffed with elongated semilunar erythrocytes, and the sinusoids are compressed and empty. After this, lysis of the red blood cells occurs by action of the reticuloendothelial phagocytes, and a brown pigment which gives a variable iron reaction is deposited in these cells. The extravasations of blood begin to organize, with the formation of siderotic nodules containing multinuclear giant cells of the foreign body type. Some of the smaller blood vessels may be surrounded by inflammatory cells and contain thrombi, while others may be narrowed by hyperplasia or hyalinization of the subintimal layer. As a result of these vascular changes, small infarcts may form, which later organize without calcification or deposition of pigment. The larger splenic arteries, however, do not contain any thrombi, nor are any large infarcts observed in sickle cell disease as a rule.

We have seen a case of sickle cell disease in which severe hemolytic anemia developed and which exemplifies the characteristic changes in the early stages.

REPORT OF CASE 1

A white boy born in New York, of Greek parentage, was admitted to a hospital in a dying condition. A clinical history was not obtainable, and the case

4. Yater, W. M., and Mollari, M.: J. A. M. A. **96**:1671, 1931.
5. Haden, R. L., and Evans, F. D.: Arch. Int. Med. **60**:133, 1937.
6. Diggs, L. W.: J. A. M. A. **104**:538, 1935.

was reported to the Office of the Chief Medical Examiner because a diagnosis could not be made.

Necropsy (thirty hours after death).—The body was that of a white boy of 6 years. The height was 122 cm. and the weight 19.5 Kg. The features were not negroid but characteristic of a dark Mediterranean race. The body was slender, with slight muscular development and poor nutrition, and the skin and scleras were pale without showing jaundice.

On section the blood was scant, purplish red, thin and watery. The organs were pale, especially the heart, lungs and brain tissue. The inside of the left subdural space was lined with a thin hemorrhagic membrane. The lymph nodes were slightly enlarged. The liver and kidneys were dark reddish brown but otherwise normal.

The spleen was enlarged, with a thin capsule and a smooth outer surface. It was tense and rubbery. The weight was 450 Gm. The cut surface was velvety in appearance and raspberry red and bulged outward slightly. The malpighian bodies were of pinhead size and widely separated. The septums were difficult to distinguish in the swollen pulp.

Microscopic Examination.—Sections were stained with hematoxylin and erythrosin, sudan III and an iron stain. The spleen showed small malpighian bodies, each containing a normal central artery surrounded by normal lymphoid cells without germinal centers. The capillaries in these bodies were distended with elongated semilunar erythrocytes, some of which were shaped like slippers and billiard cues (fig. 1*A*). The pulp was stuffed with cells of the same type, and the sinusoids were obscured. A few siderotic nodules were seen containing granular brown pigment which gave a variable iron reaction, tangled filamentous fungoid structures which were iron staining, numerous multinucleated foreign body giant cells and thick-walled, sclerotic arterioles the walls of which were permeated with iron pigment. The trabeculae were normal except in places where they showed a little thickening and hyaline change.

The sinusoids of the liver were stuffed with tangled masses of elongated sickle cells. There was moderate fatty infiltration of the parenchyma cells.

The glomeruli of the kidneys were engorged with normal red cells and sickle cells. There were brown pigment granules in the cells of the convoluted tubules, which gave a variable iron stain.

The marrow was hyperplastic but otherwise natural. The other organs were natural.

The diagnosis of sickle cell disease was not made at the time of the necropsy, although it was recognized then that death was due to severe anemia with deformed erythrocytes. It was not until several years later, when the slides were reexamined, that the true condition was identified.

Case 1 offers a good example of the pathologic changes that occur in acute exacerbations of the early stages of the disease.[7] It is also unusual in that the condition was discovered in a white person. In the few cases described in which the patient was of the white race the

7. (*a*) Wollstein, M., and Kreidel, K. W.: Am. J. Dis. Child. **36**:998, 1928. (*b*) Ryerson, G. S., and Terplan, K. L.: Folia haemat. **53**:353, 1935.

nationalities were as follows: Greek (by descent) [8]; Italian or Sicilian (by descent) [9]; a white American, without Negro or Mediterranean intermixture.[10] In most of the cases of sicklemia and sickle cell disease reported in the United States the patients have been Negroes, and inves-

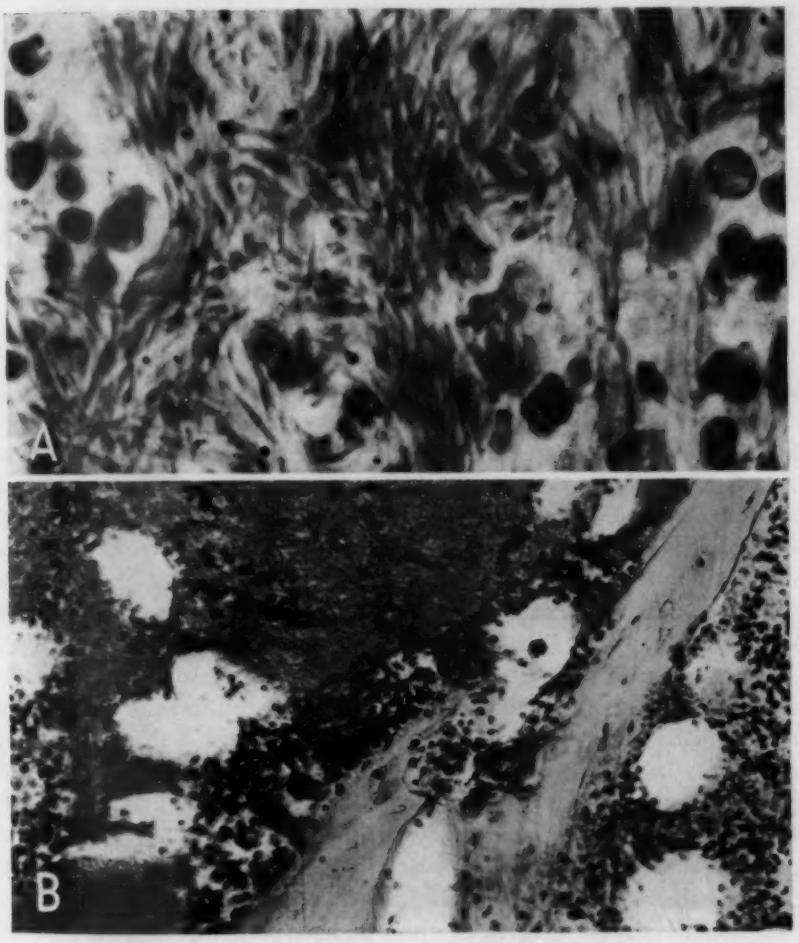

Fig. 1.—*A* (case 1), tangled masses of elongated erythrocytes in the blood channels of a splenic follicle; high power. *B* (case 2), hemorrhages in necrotic marrow of a lumbar vertebra; low power.

8. (*a*) Cooley, T. B., and Lee, P.: Am. J. Dis. Child. **38**:103, 1929. (*b*) Wade, L. J., and Stevenson, L. D.: Am. J. Path. **17**:47, 1941.

9. (*a*) Pincus, J. B., and Rosenfeld, S.: Am. J. M. Sc. **184**:674, 1932. (*b*) Clarke, F.: Nebraska M. J. **18**:376, 1933. (*c*) Wiener, S. A.: J. Mt. Sinai Hosp. **4**:88, 1937. (*d*) Greenwald, L., and Barrett, J. B.: Am. J. M. Sc. **199**:768, 1940. Haden and Evans.[5]

10. Cooke, J. V., and Keller, M. J.: J. Pediat. **5**:601. 1934.

tigators estimate that about 7.5 per cent of that race exhibit the anomaly. In a few cases Negroes from other parts of the world have been found to have the anomaly, but not enough data are available to enable one to judge the frequency of the occurrence and the extent of the distribution of the condition over the globe. Most reports on both Negro and white patients indicate that sicklemia is a familial and hereditary trait transmitted as a dominant characteristic.[11] Usually, when families of persons with the condition are investigated, some of the relatives are found to have sickle cells in the blood.

Sickle cell disease that runs a more chronic course is characterized by progressive organization of the hemorrhagic extravasations in the spleen. The contraction of the newly formed fibrous tissue causes the spleen to become gradually firmer, smaller and more nodular. The capsule is irregularly thickened, and the trabeculae are prominent. Numerous siderotic nodules with pigment granules and brown filaments which give the reactions for iron and calcium are attached to the trabeculae. Some of these filaments are in tangled masses, while others are arranged in parallel bundles along the axis of the blood vessels. Numerous foreign body giant cells may be present around the granules and the filaments. Later the pulp is replaced by fibrous tissue, and the spleen is finally converted into a hyaline mass, in which the pigment and the incrustations of iron and calcium are embedded. A few capillary spaces and small pools of blood containing sickle-shaped erythrocytes are scattered here and there. All normal structure of the spleen is destroyed, and the organ shrinks to an atrophic nodular mass weighing only a few grams.[6]

Changes also occur in the bones and marrow. In the early stages the red marrow is cellular and congested; later, hemorrhages occur between islands of regenerating cells.[12] Nucleated sickle-shaped red blood cells have been described by some. Thromboses, necroses, crystalline pigment deposits and sclerotic changes in the marrow and bony tissue have been reported. Osteoporosis may be present in the skull and may give a characteristic roentgen picture.[13]

The other organs are not changed characteristically as a rule, but a few cases have been reported in which unusual pathologic lesions occurred as a result of sickle cell disease. The patient in case No. 2 of Ryerson and Terplan [7b] had acute yellow atrophy of the liver; Bauer [3] noted extensive cortical necroses of both kidneys in one of his cases; Bridgers [14]

11. Huck, J. G.: Bull. Johns Hopkins Hosp. **34**:335, 1923. Steinberg.[2] Cooley and Lee.[8a] Pincus and Rosenfeld.[9a] Clarke.[9b]

12. Diggs, L. W., and Ching, R. E.: South. M. J. **27**:839, 1934.

13. Diggs, L. W.; Pulliam, H. N., and King, J. C.: South. M. J. **30**:247, 1937.

14. Bridgers, W. H.: Am. J. Path. **15**:353, 1939.

reported endarteritis of the cerebral vessels, and Yater and Hansmann [15] described sclerosis and thrombosis of the pulmonary arteries.

The case of Wade and Stevenson [8b] was that of a white woman of Greek descent in whom fatal fat embolism developed as a complication of sickle cell anemia. Her final illness started with a severe pain in the lumbar vertebrae about fourteen days prior to death; shortly after this, petechial hemorrhages appeared in the conjunctivas and the skin, and the patient gradually became comatose. Death occurred in an attack of dyspnea, with the patient still in deep coma. Necropsy disclosed a sickle cell anemia with splenomegaly, accompanied by necroses and sidero-fibrotic nodules, focal necroses of the marrow and fatal fat embolism in the lungs, kidneys, spleen and brain. The fat embolism was considered to be a possible result of the necrotic changes in the marrow. Up to the time of this report, no similar case had been reported in the literature.

We have seen a case of fat embolism complicating sickle cell disease which has many resemblances to the case of Wade and Stevenson.[8b]

REPORT OF CASE 2

A Negro woman of 49 years was admitted to a hospital in deep coma. Her husband stated that she had been in good health until two days prior to admission, when she complained of a severe pain low in the back. At that time she took an unknown quantity of acetylsalicylic acid and barbiturate tablets. Twenty-four hours later a physician was summoned, and he gave her a hypodermic injection of some unknown substance. Fifteen minutes after that she became increasingly stuporous and remained in that state up to the time of her admission to the hospital on the following day.

The patient was in deep coma, with reflexes depressed but equal on both sides, with pupils small but reacting to light, and with slight response to strong supra-orbital pressure. At that time the temperature was 102 F., the pulse rate 98, the respirations 26 and the blood pressure 120 systolic to 80 diastolic.

Five hours after admission the respiratory rate was increased to 40, and one hour after that the patient breathed heavily and began to froth at the mouth. Just prior to death the temperature was still 102 F., the pulse rate 130 and the blood pressure 100 systolic with 80 diastolic. The respiratory rate was not recorded for this period. The patient became incontinent of urine and died in deep coma twelve hours after admission.

Necropsy (eighteen hours after death).—The body was that of an obese Negro woman. The height was 142 cm. and the weight about 57 Kg. The mucous membranes were pale, and there was a slight icteric tinge to the scleras.

The lungs were salmon pink, well aerated and moderately edematous. Both lungs together weighed 720 Gm. A few dark red irregular areas of hemorrhage, 1 to 1.5 cm. in diameter, were present here and there in the lung tissue.

The spleen was a firm yellowish white mass, weighing 30 Gm., and was adherent to the surrounding structures. On cut section it presented a piebald appearance, as

15. Yater, W. M., and Hansmann, G. H.: Am. J. M. Sc. **191**:474, 1936.

irregular raspberry red areas 1 to 5 mm. in diameter and indistinct grayish brown areas of about the same size and shape were interspersed throughout the yellowish white hyaline connective tissue.

The marrow in the lumbar vertebrae was a dull brick red speckled with small dark red areas of hemorrhage about 0.5 mm. in diameter. The bone marrow in the rib and in the lower end of the right femur was dark red.

The liver was large, reddish brown and firm. The edges were rounded. The cut surface was hemorrhagic and velvety in appearance.

The dural veins were engorged, and the vessels of the brain were markedly injected.

The other organs did not show any obvious lesions on macroscopic examination. There was no sign of trauma.

Microscopic Examination.—Blood scraped from the cut surface of the spleen was examined both in wet smears and in dry smears stained with Giemsa stain. Semilunar erythrocytes were demonstrated in both examinations.

Specimens of the tissues were fixed in 10 per cent formaldehyde solution and stained with hematoxylin and erythrosin, sudan III, and iron stain, Marchi's stain and Masson's [16] trichrome stain.

All normal splenic structure had disappeared. The principal tissue was a hyalinized connective tissue, in which were groups of capillary vessels and small pools of blood containing semilunar erythrocytes. Scattered among these were collections of dark blue-staining fibrils of fungoid type, arranged in parallel fashion or in interwoven clumps, and also collections of square translucent yellowish brown crystals; both these structures gave the iron reaction. A few groups of lymphoid cells were present here and there but could not be identified as splenic follicles. The smaller arteries were arranged irregularly in the hyaline tissue, and some of them were sclerotic.

There was normal cancellous bone in the bodies of the lumbar vertebrae. The marrow in between was distributed around a moderate number of adipose cells, but its structure was much altered. The cellular components were scattered and surrounded by abundant formless cellular debris, and only in a few places were the marrow cells collected in small groups. Except for an occasional megakaryocyte, the various types of marrow cells could not be identified with certainty, as most of them showed necrotic changes in the form of pyknotic nuclei and eosinophilic cytoplasm. Multiple small hemorrhages and pools of blood were present in between the necrotic cells and the depot fat cells. There was iron-staining material in the bone marrow. Sections of the marrow of the ribs and of that of the shaft of the femur disclosed similar changes (figs. 1 *B* and 2 *A*).

The sinusoids of the liver were engorged with tangled clumps of elongated erythrocytes (2 *B*). The parenchyma cells were normal. A few miliary tubercles were seen here and there.

Sections of the brain disclosed an insignificant infiltration of lymphoid cells around the main stem of the basilar artery and also around a few of its intrapontile branches. The Virchow-Robin spaces around some of the pontile vessels were markedly distended with fluid. Elsewhere in the brain the vessels appeared natural. Abnormal hemorrhages and areas of necrosis were not found in the brain substance. A moderate number of sausage-shaped fat emboli were observed in the arterioles of the cerebral cortex.

The myocardium was normal, but here and there in some of the smaller arterioles were a few fat emboli.

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16. Masson, P.: J. Tech. Methods **12**:75, 1929.

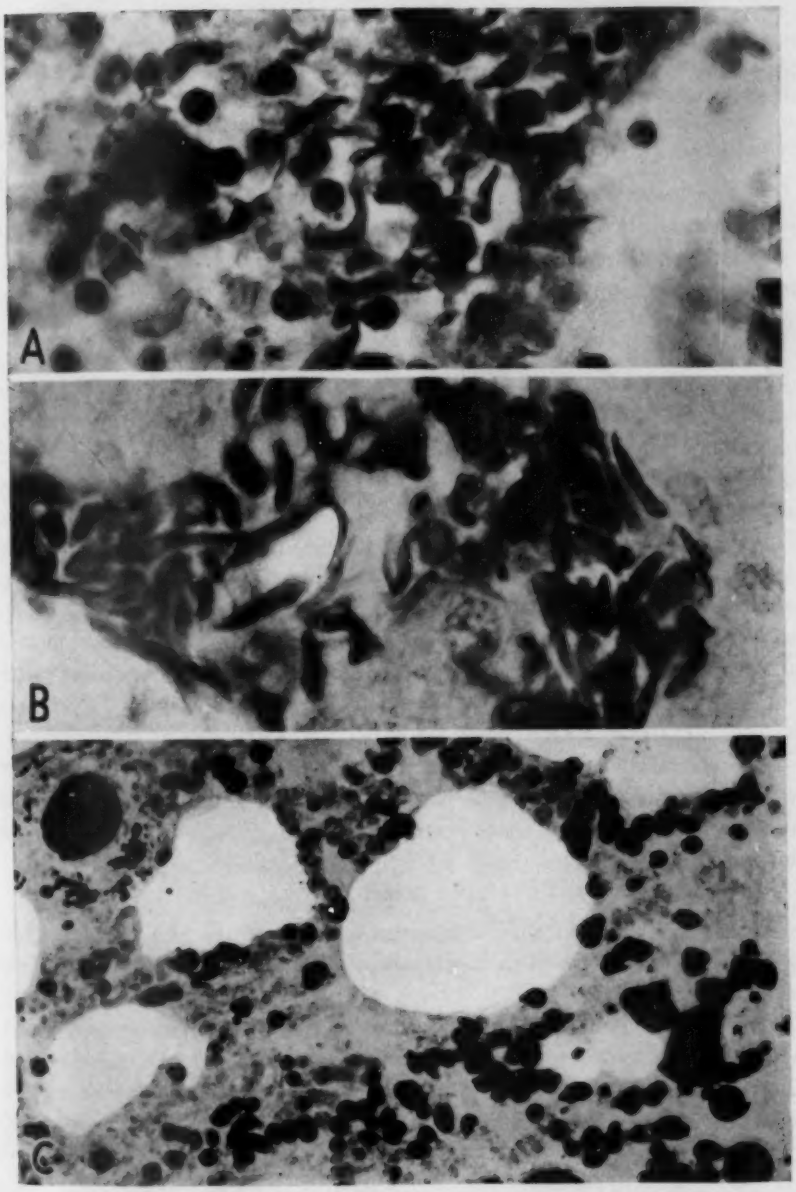

Fig. 2 (case 2).—*A*, sickle-shaped erythrocytes in necrotic marrow of the lower end of the right femur; high power. *B*, tangled masses of sickle-shaped erythrocytes in the sinusoids of the liver; high power. *C*, marked fat embolism of the arterioles and capillaries of the lung: Marchi stain; low power.

Numerous sausage-shaped and coiled fat emboli were present in the glomeruli of the kidneys, but otherwise these organs were normal.

The pulmonary alveoli contained a little edematous fluid, and those in the hemorrhagic areas also contained semilunar erythrocytes. A few miliary tubercles were present here and there in the lung tissue. Fat stains disclosed in the pulmonary circulation fat embolism in sufficient amount to cause congestion of the arterioles and capillaries with spherical and ovoid globules so that the alveolar walls were outlined (fig. 2 *C*).

The anatomic diagnosis was: sickle cell disease with splenic atrophy; necrosis and hemorrhages of the marrow; pulmonary fat embolism, with fat emboli in the kidneys, cerebral cortex and myocardium; marked passive congestion of the brain; slight miliary tuberculosis of the lungs and liver.

Our patient, like the one whose case was reported by Wade and Stevenson,[8b] complained of a severe pain in the back just prior to the onset of the final symptoms, which were referable to a fatal attack of pulmonary fat embolism. The lesions of the marrow, produced by the sickle cell disease, especially those in the marrow of the lumbar vertebrae, undoubtedly explain the pain in the back and the fatal complication. The changes in the marrow substance include the formation of a necrotic debris containing fatty material, broken blood vessels with numerous small hemorrhages and a rise of internal pressure in the bones themselves. In combination they are predisposing factors in the development of severe fat embolism and are capable of causing this condition, as in our case. Such an interpretation is supported by many references in the literature, which have described hemorrhages in the marrow, both from traumatic and from nontraumatic causes, as the etiologic factor in the production of fat embolism.[17] However, aside from the case of Wade and Stevenson,[8b] there are not any cases reported which resemble our case.

SUMMARY

Sickle cell disease with severe hemolytic anemia and splenomegaly is described as observed in a white boy of 6 years, born in New York of Greek parentage.

Sickle cell disease with atrophic spleen and bone marrow permeated with necroses and hemorrhages is reported as studied in a middle-aged Negro woman of 49 years. A fatal pulmonary fat embolism developed from the lesions in the marrow.

17. Vance, B. M.: Arch. Surg. **23**:426, 1931.

EXPERIMENTAL RICKETS

BLOOD AND TISSUE CHANGES IN PUPPIES RECEIVING A DIET VERY LOW IN PHOSPHORUS, WITH AND WITHOUT VITAMIN D

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The objects of the studies here reported were to obtain repeated observations of the changes in puppies when experimental rickets was induced by a diet very low (0.024 per cent) in phosphorus and to determine the effects of the presence or absence of vitamin D in the control and experimental diets. Diets similarly low in phosphorus, in contrast to the usual low phosphorus (0.1 to 0.3 per cent) rachitogenic diets, were fed to rats by Jones,[1] by Schneider and Steenbock [2] and by Day and McCollum.[3]

The advantage of using puppies for the observation of changes in the blood over long periods has not been adequately exploited with regard to experimental rickets, and the natural history of this condition is consequently deficient in many respects. We have followed the changes in the blood and at the same time made roentgenographic observations of the development of florid rickets over a period of one hundred days. The animals were then killed, and the soft tissues and bones were examined histologically, the latter in undecalcified sections stained positively for bone salt. The state of calcification in the bones was correlated with the solubility products of the secondary and tertiary phosphates of calcium and the concentrations of calcium and phosphate

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Dr. R. B. Lewis of the department of radiology of Northwestern University Medical School made the roentgenograms of the animals, and Dr. Francis D. Gunn of the department of pathology made the histologic examination of the parathyroids and other soft tissues.

1. Jones, J. H.: J. Nutrition **17**:601, 1939.
2. Schneider, H., and Steenbock, H.: J. Biol. Chem. **128**:159, 1939.
3. Day, H. G., and McCollum, E. V.: J. Biol. Chem. **130**:269, 1939.

ions in the serum. Chemical and histologic examinations of the bones were also made for the amount and distribution of phosphatase.

EXPERIMENTAL GROUPS AND PROCEDURES

Fifteen mongrel puppies born in the laboratory were weaned at approximately 6 weeks of age to a diet of milk and cooked meat. This was supplemented every other day by 2,800 U. S. P. units of vitamin A and 400 U. S. P. units of vitamin D in the form of percomorph liver oil until they were 10 weeks old. At this time they were put on the control experimental diet for two weeks, then divided into four groups and fed the experimental diets. The animals were caged separately; the floors of the cages were raised and made of no. 2 mesh screening. Each cage was scrubbed daily with soap and dilute saponated solution of cresol.

TABLE 1.—*Plan of Experiment and Growth of Puppies During Its Duration*

Group	Animal *	Diet	Vitamin D, U. S. P. Units per Kg.	Initial Weight, Kg.	Final Weight, Kg.	Daily Gain, Group Average, Kg.†
I	A_1	Control.....	50	2.9	7.9	
	A_3	Control.....	50	2.6	7.7	
	D_{18}	Control.....	50	4.2	14.0	0.074
II	A_2	Control.....	None	2.7	....	
	A_7	Control.....	None	4.0	12.5	
	C_{13}	Control.....	None	3.3	10.0	
	B_{10}	Control.....	None	4.8	10.0	0.060
III	A_3	Low phosphorus.....	50	3.1	5.8	
	A_5	Low phosphorus.....	50	3.5	...	
	C_{17}	Low phosphorus.....	50	3.9	7.0	
	B_{11}	Low phosphorus.....	50	2.4	5.0	0.027
IV	A_4	Low phosphorus.....	None	2.7	5.0	
	A_6	Low phosphorus.....	None	4.0	8.0	
	A_9	Low phosphorus.....	None	3.2	6.7	
	C_{12}	Low phosphorus.....	None	4.0	7.2	0.032

* Litter mates have the same letter.
† Dogs A_2 and A_5 are excluded because they died before the completion of the experiment.

The experimental groups and the general plan of the experiment are shown in table 1; for the most part the data are reported as averages for the groups, but the animals are identified individually and by litters.

The experiment lasted one hundred days. The puppies were bled at the beginning of the experiment and on the seventeenth, thirty-second, forty-fourth, sixtieth and hundredth days. The blood serum was analyzed for calcium, inorganic phosphorus and phosphatase, and the whole blood, for cell volume, total acid-soluble phosphorus and inorganic phosphorus. Roentgenograms of the foreleg were taken every two weeks during the experiment.

Two animals from each group were killed at the end of one hundred days, after the final bleeding. Bones and soft tissues were preserved for histologic examination, and the phosphatase content of a costochondral junction and of the renal cortex was determined.

Diets.—The diets were made up twice weekly and stored in a refrigerator in closed containers.

The control diet[4] consisted of: sucrose, 57.5 Gm.; lard, 30 Gm.; cellulose (Woodward and McCay[5]), 5 Gm.; Wesson's salt mixture (Wesson[6]), 5 Gm.; liver extract, 2 Gm.; choline chloride, 0.5 Gm.

To each 100 Gm. of this mixture were added 10 mg. of nicotinic acid and 1 mg. each of thiamine hydrochloride, riboflavin and pyridoxine. Vitamin A was added as carotene in oil, each animal receiving 50 U. S. P. XI units per kilogram per day. Powdered white of egg, 8.8 Gm. per kilogram of body weight, was dissolved in distilled water, thoroughly coagulated on a steam bath and mixed with the daily allowance (initially 33 Gm. per kilogram of body weight) of the basal mixture. This diet furnished approximately 198 calories per kilogram of body weight. The animals receiving vitamin D were given 50 U. S. P. XI units per kilogram as viosterol in oil, administered daily from a dropper. All animals were allowed distilled water unrestrictedly.

The diet deficient in phosphorus was identical in every respect with the control diet except that the phosphate-containing salts (KH_2PO_4 and $Ca_3[PO_4]_2$) were omitted from the salt mixture, and the potassium chloride and calcium carbonate were correspondingly increased. Of this altered mixture, 3.92 Gm. was used in 100 Gm. of the basal diet, the cellulose content of the latter being increased to 6.08 Gm. By analysis the phosphorus-deficient basal diet was shown to contain 8.24 mg. of phosphorus per hundred grams, and the egg white to contain 88 mg. per hundred grams. The diet as fed consisted of 79 per cent of the basal mixture and 21 per cent of powdered egg white; the complete ration contained 0.024 per cent phosphorus. The amount of this diet initially offered to the animals, 41.8 Gm. per kilogram of body weight, provided a phosphorus intake of 10.45 mg. per kilogram. However, this amount was greater than that actually consumed.

The control basal mixture contained 508 mg. of phosphorus per hundred grams. With the same proportions of basal mixture and egg white as before, the control ration contained 0.42 per cent of phosphorus and provided 176 mg. per kilogram of body weight. Assuming that the salt mixture was the only source of calcium, all the diets contained 0.56 per cent of calcium and provided for an intake of 233 mg. per kilogram of body weight.

After the third bleeding (thirty-second day of the experiment) the amount of salt mixture in the diet of all groups was reduced by one half, and the sucrose content increased accordingly. At the same time the food offered each animal was reduced by one quarter. These changes in the diet were occasioned by a progressive reduction in food intake and marked hypercalcemia, possibly of alimentary origin, in all the animals in groups III and IV.

Analytic Procedures.—Serum calcium determinations were made in duplicate (Clark and Collip).[7] Serum phosphatase was determined according to the method

4. Dr. David Klein of the Wilson Laboratories supplied liver extract; Dr. D. F. Robertson of Merck & Company, Inc., pyridoxine and choline chloride; Dr. Evans McChesney of the Winthrop Chemical Company, Inc., thiamine hydrochloride, riboflavin and nicotinic acid; the S. M. A. Corporation, carotene in oil, and Mead Johnson & Company, viosterol in oil.

5. Woodward, J. W., and McCay, C. M.: Proc. Soc. Exper. Biol. & Med. **30**:241, 1932.

6. Wesson, L. G.: Science **75**:339, 1932.

7. Clark, E. P., and Collip, J. B.: J. Biol. Chem. **63**:461, 1925.

of A. Bodansky,[8b] and inorganic phosphorus, according to the Kuttner-Lichtenstein method as modified by A. Bodansky.[8a] Tissue phosphatase was determined by the method of O. Bodansky.[9] Colorimetric readings were taken with a Klett-Summerson photoelectric colorimeter using filter 54; comparable standards were assayed simultaneously with the unknown. All blood samples were drawn from an external jugular vein into an oiled syringe with a minimum of stasis, while the animals were fasting. Only an occasional serum showed slight hemolysis. All determinations ware made immediately, those on whole blood receiving attention first. Potassium oxalate and sodium fluoride, 2.5 mg. of each per cubic centimeter of blood, were used as an anticoagulant for the whole blood determinations. It was possible to analyze the blood and serum from half the dogs in one day. The inorganic and organic acid-soluble phosphorus were determined on whole blood after hemolysis with distilled water and removal of protein with trichloroacetic acid. Hematocrit determinations on the same blood sample were made by centrifuging a 10 cm. column of blood in a 2 mm. bore capillary tube for thirty minutes at a speed of 2,000 revolutions per minute.

RESULTS

Gross Results of Deficiency.—The growth obtained during the hundred days of the experiment is indicated in table 1. This table shows that the animals receiving the control diet grew normally while those receiving the low phosphorus diet grew at a much reduced rate. The slightly better growth of the animals not given vitamin D we do not consider significant. In all the animals to which we administered the low phosphorus diet florid rickets developed whether or not they were given vitamin D. Roentgenologically, the first definite manifestations of rickets were observed four weeks after the beginning of the experiment. None of the roentgenograms showed any evidence of healing.

Anorexia, lassitude, pronated feet and lack of muscle tone became conspicuous in all animals on the low phosphorus diet. Some of these animals refused to stand on their four feet after the first three or four weeks of the experiment while others intermittently stood and walked about throughout the entire experiment. There was a corresponding difference in the gross deformities. Some of the animals showed little bowing of the forelegs; in others this condition was acute. The weakness or unwillingness to stand which several animals manifested may have contributed to the malnutrition and debility which became apparent as the experiment progressed. In one animal the joints became very tender; the ascorbic acid content of the serum of this animal was normal

8. Bodansky, A.: (*a*) J. Biol. Chem. **99**:197, 1932; (*b*) **101**:93, 1933.

9. Bodansky, O.: J. Biol. Chem. **114**:273, 1936.

for a dog. Defects of occlusion due to a steadily increasing protrusion of the lower jaw developed in practically every animal of groups III and IV, in one animal (B_{10}) of group II but in none of group I.

The animals receiving the low phosphorus diet showed a constant increase in the mobility of the thoracic cage, as in the rats described by Day and McCollum,[3] but the exudation and crusting about the nasal orifices which these authors described were not present in our animals. An increased rate of respiration was apparent as well as an acute angulation with the formation of a sulcus (Harrison's groove) at the site of attachment of the diaphragm to the ribs. As a result of this deformity the capacity of the thorax was reduced, and the extent of beading of the ribs could not be accurately evaluated from external palpation.

Two animals died during the experiment. The death of puppy A_2 was preceded by persistent diarrhea; the cause of death was not ascertained at autopsy. Puppy A_8, receiving the low phosphorus diet with vitamin D, died a few days after we noted a serum calcium value of 20.6 mg. per hundred cubic centimeters, presumably from the effects of hypercalcemia.

Serum Calcium.—The data on serum calcium are presented in figure 1. The curves for the controls (groups I and II) are almost identical, while the averages of group III, receiving the low phosphorus diet plus vitamin D, appear significantly higher than those of group IV, receiving the same diet without vitamin D.

Hypercalcemia developed in all animals of groups III and IV, reaching its peak on the thirty-second day of the experiment. At this time the salt content of the diet was reduced to one-half its former value, and the food offered to each animal was reduced to three fourths the former amount. The hypercalcemia gradually declined, the serum calcium values of all animals being within the normal range at the termination of the experiment.

Inorganic Phosphorus.—The serum inorganic phosphorus (fig. 1) of the control groups I and II showed relatively little change throughout the experiment although there was a slight decline, particularly in the group receiving no vitamin D (II), as the experiment progressed. Groups III and IV (low phosphorus) both showed a distinct reduction in the inorganic phosphorus of the serum with practically identical curves for the two groups. The values for individual members of the low phosphorus groups were likewise almost identical. After the first three determinations (thirty-two days) there was practically no change,

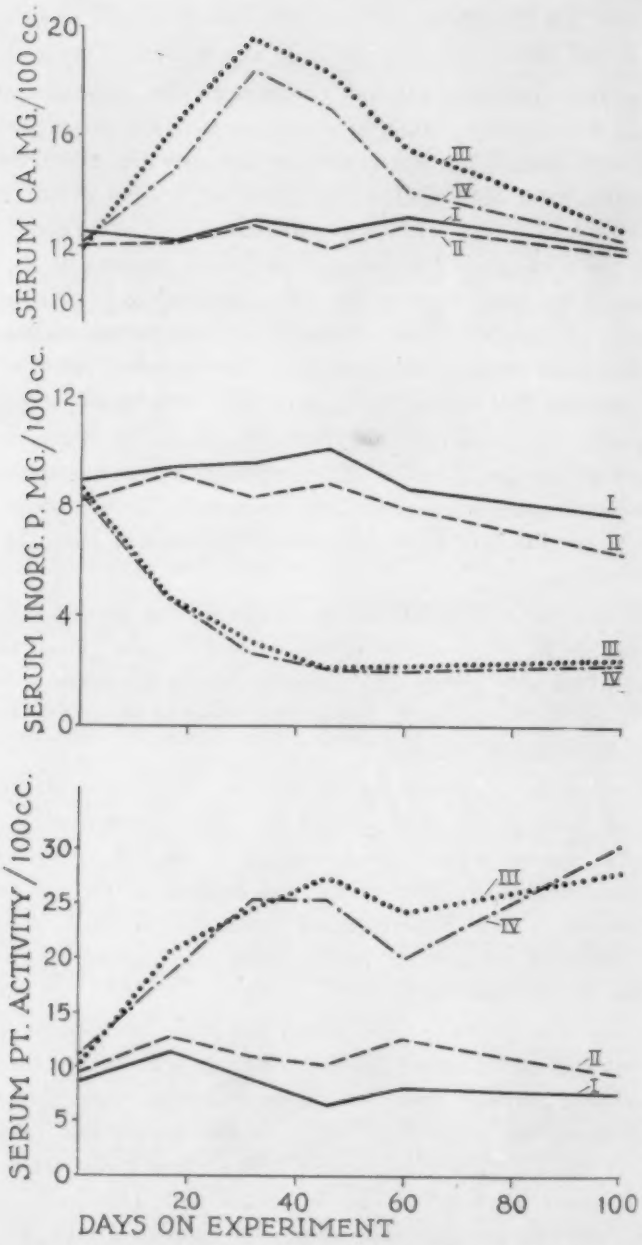

Fig. 1.—The effects of a low phosphorus diet with and without vitamin D on the serum calcium, inorganic phosphorus and phosphatase of puppies. Groups III and IV received a diet very low in phosphorus; groups I and II, a diet adequate in phosphorus. Groups I and III were given vitamin D.

and the subsequent values for these animals were practically constant and identical. The inorganic phosphorus values for whole blood are not presented because of their similarity to those obtained from serum; they were nearly always slightly lower than the latter.

Acid-Soluble Organic Phosphorus.—The organic acid-soluble phosphorus of whole blood (fig. 2) decreased in all four groups during the first two weeks of the experiment. Thereafter this phosphorus fraction remained relatively constant for groups I and II, while groups III and IV showed a further slight decrease. The results are little altered by correcting for variations in cell volume. The decrease of the acid-soluble organic phosphorus in the animals given the low phosphorus diet was much less definite than the changes in the inorganic phosphorus of either serum or whole blood.

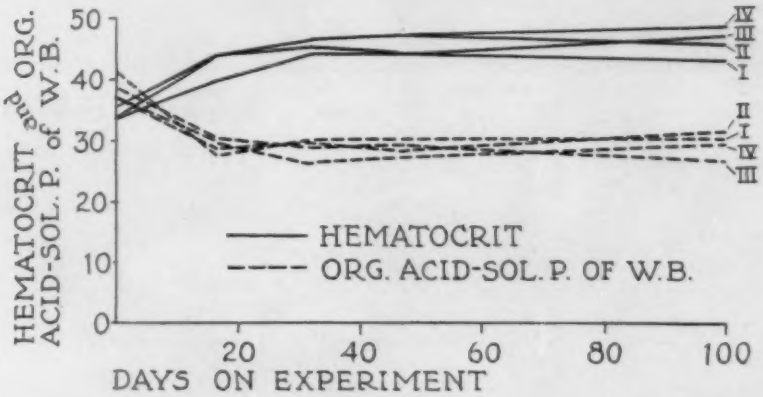

Fig. 2.—The effects of a low phosphorus diet with and without vitamin D on the cell volume and organic acid-soluble phosphorus of whole blood. Groups III and IV received a diet very low in phosphorus; groups I and II, a diet adequate in phosphorus. Groups I and III were given vitamin D.

Hematocrit Values.—These were similar in all groups (fig. 2). There was an initial increase in cell volume after which the readings were all relatively constant, though slightly higher for groups III and IV (low phosphorus).

Serum Phosphatase.—All of the serum phosphatase values (fig. 1) for the control groups I and II were in close agreement, except in the case of an animal in group II (C_{12}) which consistently maintained a higher phosphatase level throughout most of the experiment; however, its phosphatase level was never as high as that of the groups receiving the low phosphorus diet. The serum phosphatase of groups III and IV (low phosphorus) increased to approximately three times the initial

level; the changes in all these animals were of similar magnitude and were uninfluenced by the presence or absence of vitamin D.

Tissue Phosphatase.—Determinations of phosphatase were carried out on the costochondral junction and on the renal cortex of 2 animals from each group, killed at the end of the experiment. These results are shown in table 2. The animals of group I had somewhat lower bone phosphatase values than those of the other three groups. The phosphatase values for the renal cortex were too variable to permit any definite interpretation.

HISTOLOGIC OBSERVATIONS

Histologic examinations of the soft tissues and bones were made on material from 2 representatives of each of the four groups. Liver, kidney, pancreas and thoracic aorta were examined after staining with

TABLE 2.—*Phosphatase Content of Costochondral Junction and Renal Cortex*

Group	Animal	Bone Phosphatase *	Renal Phosphatase *
I	A_1	14.2	50.8
	A_3	14.7	38.8
II	B_{10}	18.8	50.1
	C_{12}	17.4	37.4
III	C_{17}	19.8	46.9
	B_{11}	15.1	41.1
IV	A_4	16.8	45.6
	C_{13}	17.9	21.1

* The amounts are expressed as Bodansky units of phosphatase activity per gram of fresh tissue.

hematoxylin–eosin–azure II; there were no differences between the various groups, and none of the tissues appeared definitely abnormal. The parathyroid glands were sectioned through their greatest diameters and stained with hematoxylin and eosin; there was no consistent difference in the size or appearance of the glands from the various groups.

Bones.—Sections through the costochondral junctions were made from all 8 puppies representing the four groups. Material fixed in a neutral solution containing 4 per cent formaldehyde (10 per cent concentration of solution of formaldehyde U. S. P., saturated with magnesium carbonate), embedded in nitrocellulose, cut in serial sections at 10 to 12 microns without decalcification and stained with silver nitrate and counterstained with hematoxylin and eosin by the method described by McLean and Bloom.[10] Similar material fixed in Zenker's solution (prepared with solution of formaldehyde) was embedded in nitrocellulose

10. McLean, F. C., and Bloom, W.: Anat. Rec. **78**:333, 1940.

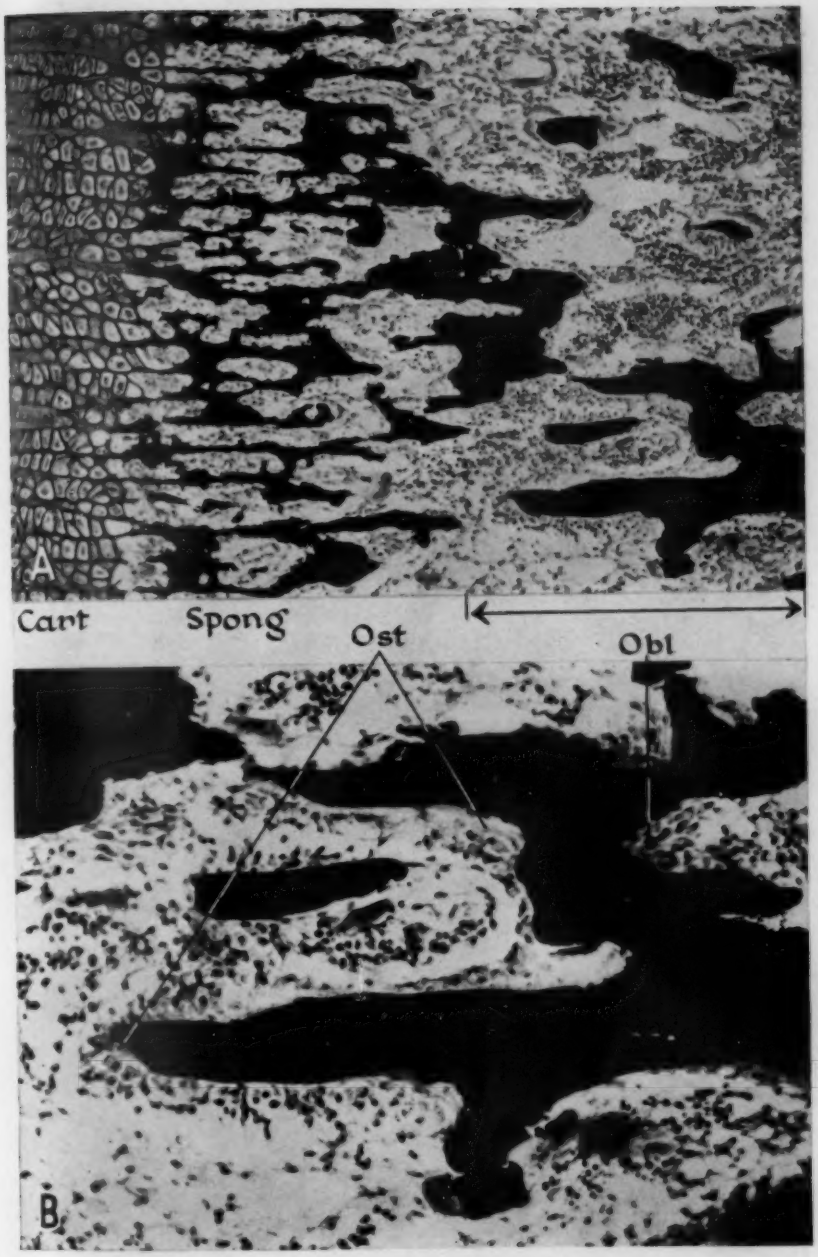

Fig. 3.—*A*, photomicrograph (× 88.5) of an undecalcified longitudinal section through a costochondral junction of control puppy A_1 of group I, fed a diet adequate in minerals and vitamin D; stained with silver nitrate, hematoxylin and eosin. *Cart* indicates cartilage and *Spong*, substantia spongiosa; vertical lines and double arrow indicate area of *B*.

B, higher power photomicrograph (× 198) of area indicated in *A*; *Ost*, osteoid borders on trabeculae of spongy bone; *Obl*, osteoblasts.

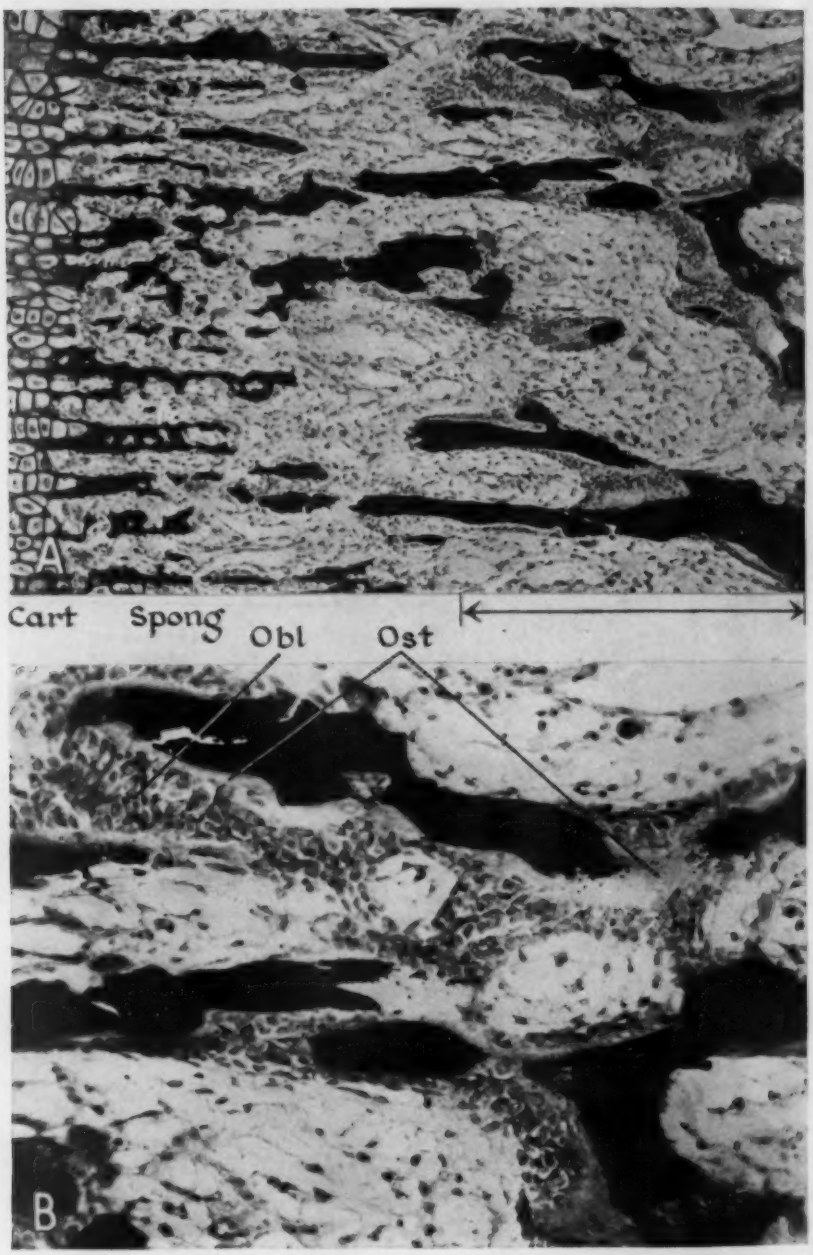

Fig. 4.—*A*, photomicrograph (× 88.5) of an undecalcified longitudinal section through a costochondral junction of puppy C_{13} of group II, fed the control diet without vitamin D; stained with silver nitrate, hematoxylin and eosin; symbols same as in figure 3.

B, higher power photomicrograph (× 198) of area indicated in *A*. Note greater prominence of osteoid borders compared with figure 3 *B*.

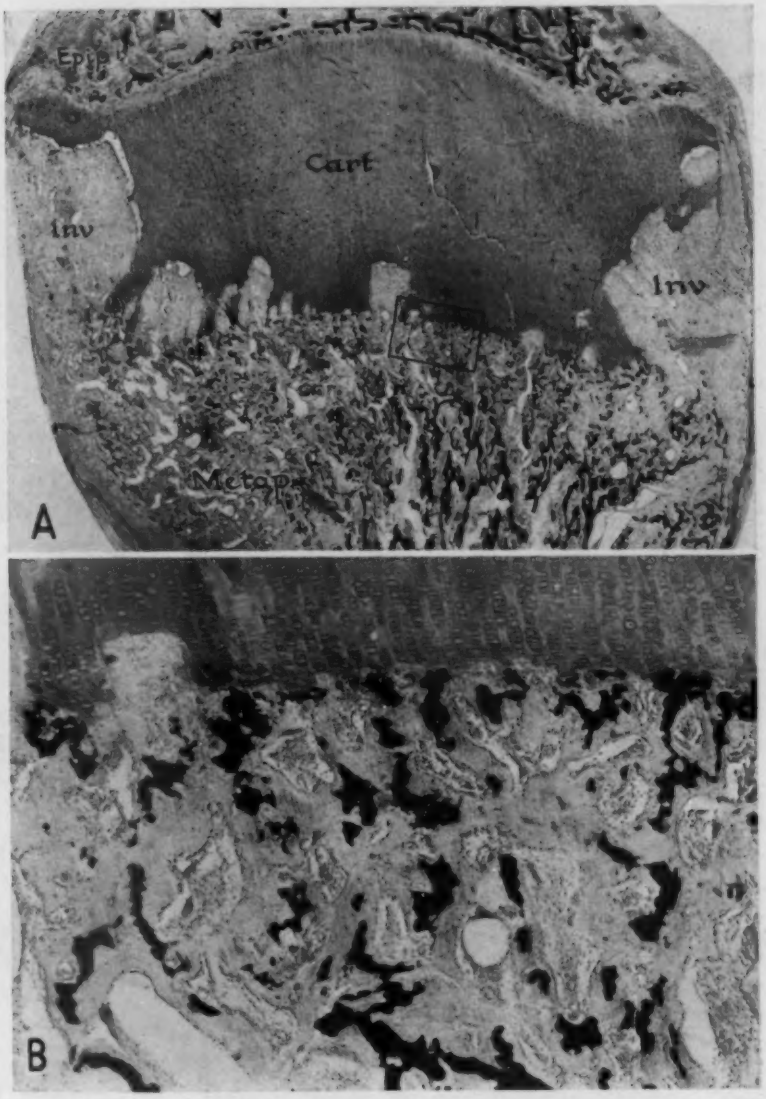

Fig. 5.—*A*, photomicrograph (× 6) of an undecalcified sagittal section through the distal end of a radius of puppy C_{17} of group III, fed the low phosphorus diet with vitamin D; stained with silver nitrate, hematoxylin and eosin. Note florid rickets with absence of calcification in the greatly thickened epiphysial cartilage plate and invasion of cartilage by osteoid tissue. *Epip* indicates epiphysis; *Cart*, cartilage; *Inv*, "bushes" of uncalcified osteoid invading cartilage; *Metap*, rachitic metaphysis.

B, higher power photomicrograph (× 51) of area in rectangle in *A*, showing junction of epiphysial cartilage with rachitic metaphysis. Note the bud of osteoid invading the cartilage, the cartilage margin being otherwise quiescent, and the trabeculae of osteoid in the metaphysis, many with cores of calcified bone.

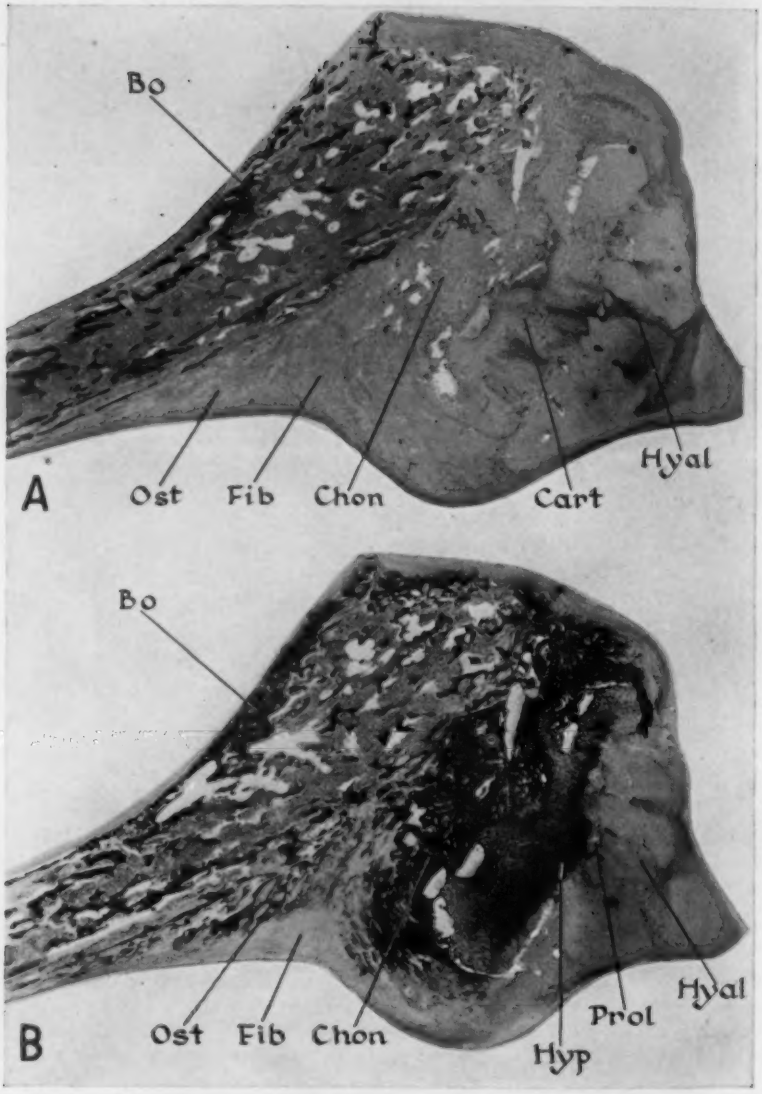

Figure 6

(See legend on opposite page)

decalcified in 3 per cent nitric acid, cut in serial sections at 10 microns and stained with hematoxylin–eosin–azure II and by the Mallory-azan method. Further, material from the distal ends of the radius and ulna from the 4 puppies from groups III and IV was similarly fixed, cut and stained.

Group I. The costochondral junctions of puppies A_1 and A_5 were those of typical normal animals in a period of rapid growth. Of particular interest were the sections stained with silver nitrate and counterstained with hematoxylin and eosin. Figure 3 illustrates such sections from puppy A_1. The sections from puppy A_5 showed identical characteristics.

Calcification extended into the cartilage for a depth of less than one cell, in many places not reaching beyond the line of removal of cartilage. The primary substantia spongiosa was free from osteoid borders for a distance of about 0.28 mm. from the cartilage. Beyond this pink-staining borders, free of bone salt and from 3 to 7 microns thick, were frequently seen in the secondary substantia spongiosa and in the shaft.

Group II. In decalcified sections stained with hematoxylin–eosin–azure II, the costochondral junctions of puppies B_{10} and C_{13} were indistinguishable from those of the puppies in group I. Striking differences, however, were revealed in the sections stained with silver nitrate, hematoxylin and eosin, illustrated from puppy C_{13} in figure 4. The findings in the sections from puppy B_{10} (fig. 7) were identical with those in the sections from puppy C_{13}.

EXPLANATION OF FIGURE 6

Fig. 6.—*A*, photomicrograph (×7) of an undecalcified section through a deformed costochondral junction of the puppy with florid rickets from which a sagittal section of a radius is shown in figure 5; alcohol fixation; silver nitrate–hematoxylin–eosin. *Bo* indicates partially calcified bone; *Ost*, uncalcified osteoid tissue; *Fib*, fibrous tissue; *Chon*, uncalcified chondro-osteoid; *Cart*, broad zone of proliferating and hypertrophic cartilage; *Hyal*, hyaline cartilage.

B, section from the same block (×7) incubated with sodium glycerophosphate in presence of calcium before staining. Silver nitrate stained both the calcified tissue and the calcium phosphate deposited where inorganic phosphate was liberated from glycerophosphate, showing localization of phosphatase. *Bo* indicates partially calcified bone, with a deposit of silver also in a layer of osteoblasts; *Ost*, trabeculae of uncalcified osteoid tissue surrounded by heavy deposits of silver in a layer of osteoblasts; *Fib*, fibrous tissue; *Chon*, chondro-osteoid heavily impregnated with silver owing to phosphatase associated with osteoblasts and with included cartilage cells; *Hyp*, hypertrophic cartilage, its matrix heavily impregnated with silver; *Prol*, proliferating cartilage; *Hyal*, hyaline cartilage, both unimpregnated.

Calcification extended into the cartilage for a depth of one cell or less, that is, about the same distance as in the puppies of group I. The primary substantia spongiosa was free from osteoid borders, which first appeared at about 0.20 mm. from the cartilage and were much more prominent than in puppy A_1. In places these osteoid borders attained a thickness of 10 to 12 microns, and in addition there were portions of newly formed trabeculae which had remained completely uncalcified (fig. 4 *B*).

Groups III and IV. All the bones examined from puppies A_4, B_{11}, C_{12} and C_{17} showed typical florid rickets, illustrated from puppy C_{17} in figures 5 and 6. No differences between groups III and IV were revealed.

The costochondral junctions were in general so badly deformed (fig. 6) that the normal relationships were much distorted. The description which follows is that of the distal end of the radius from puppy C_{17} of group IV (fig. 5). All the bones examined from the animals in the two groups had the same characteristics.

The epiphysial cartilage disk was 5 to 6 mm. thick, compared with a normal thickness of approximately 0.7 mm. For a total of approximately half its width in section, cartilage removal had ceased, and the cartilage immediately adjoined the spongy bone formed before the rachitic process was initiated. At both extremities of the section and at intervals along the junction of the cartilage with the substantia spongiosa (rachitic metaphysis) the cartilage was being invaded and replaced by uncalcified osteoid tissue in the form of "bushes," as described by Park.[11] The cartilage was free from calcification in all the sections examined from animals in these two groups.

The rachitic metaphysis consisted of thick trabeculae of uncalcified osteoid tissue formed for the most part on cores of spongy bone, presumably calcified before the rachitic process was initiated. The metaphysis was highly vascularized but contained no hemopoietic marrow.

Bone Phosphatase.—Reference has been made to the chemical determinations of phosphatase in the costochondral junctions; the results are included in table 2. Material from one costochondral junction from each of the 8 animals was fixed in 95 per cent alcohol, embedded in nitrocellulose and cut without decalcification as described. Control sections were stained with silver nitrate, hematoxylin and eosin without further treatment. Other sections were incubated with sodium glycerophosphate

11. Park, E. A., in Harvey Lectures, 1938-1939, Baltimore, Williams & Wilkins Company, 1939, p. 157.

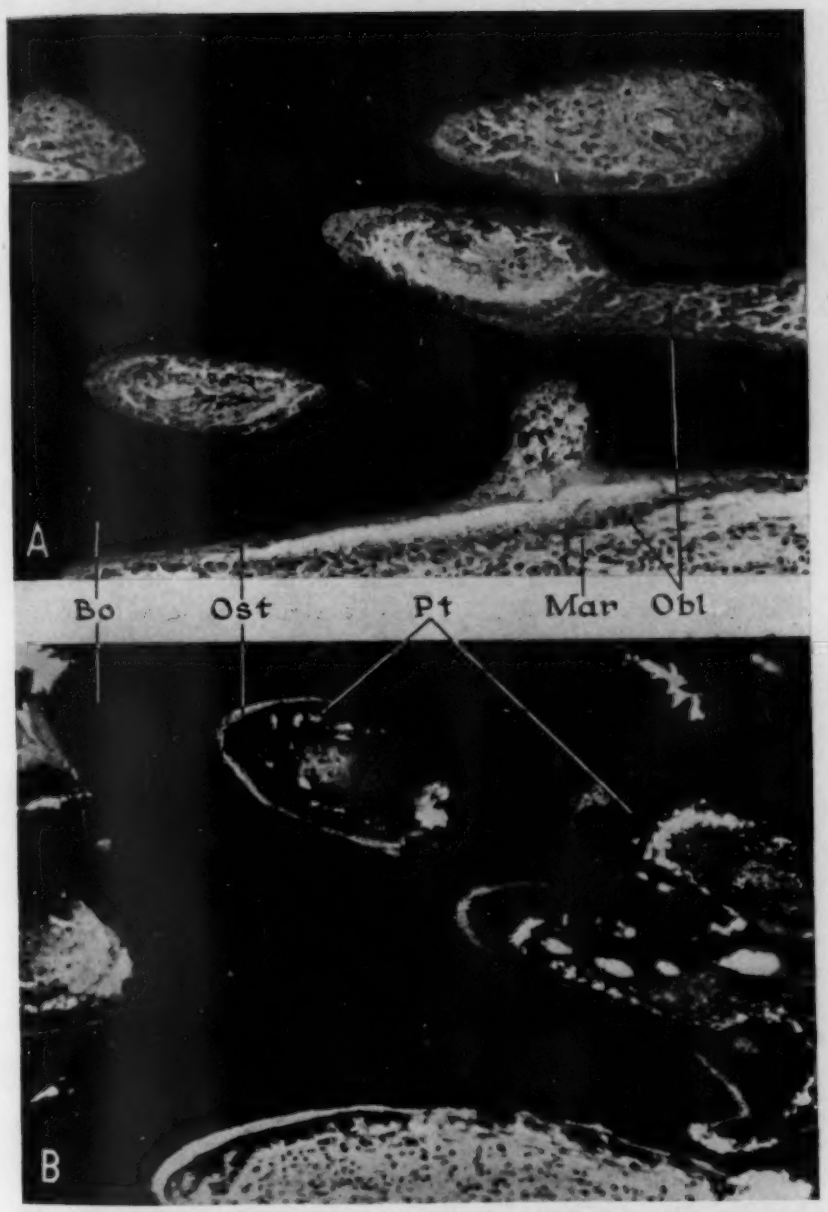

Fig. 7.—*A*, photomicrograph (× 154) of an undecalcified longitudinal section through the shaft of a rib of puppy B_{10} of group II, fed the control diet without vitamin D; alcohol fixation; silver nitrate–hematoxylin–eosin staining. *Bo* indicates calcified bone; *Ost*, borders of uncalcified osteoid tissue; *Obl*, osteoblasts; *Mar*, bone marrow.

B, similar area of a section from the same block with the same treatment as the section shown in figure 6 *B*. *Pt* indicates heavy deposits of silver in layers of osteoblasts, showing localization of phosphatase; other symbols as in *A*. Note borders of uncalcified osteoid tissue free from phosphatase.

in the presence of calcium as described by Gomori [12] and stained with silver nitrate, hematoxylin and eosin. By this method inorganic phosphate is liberated and immediately precipitated as calcium phosphate wherever phosphatase is present. The precipitate then takes the silver stain and can be distinguished from calcified bone by comparison with the control sections.

Owing to the small amount of bone salt in the bones of animals with florid rickets, the demonstration of the distribution of phosphatase in the sections from these animals was especially striking, illustrated from puppy C_{17} in figure 6. The costal cartilage was free from phosphatase up to the beginning of the zone of hypertrophic or vesicular cartilage. Beyond this line there was a deposit of silver, chiefly in the cartilage matrix and most dense in the area which would constitute the zone of provisional calcification in a normal animal.

Bone matrix was on the whole free from silver stain, which was present only in punctate areas, usually corresponding to the lacunas of included cartilage cells. It was, as a rule, not associated with osteocytes. There was a dense deposit of silver in the regions where osteoblasts were abundant, forming heavy black borders on the osteoid trabeculae, most of which had also black-staining cores of preformed bone. This deposit was so dense that its relationship to the cells was obscured. Many osteoclasts, completely free from silver, were seen, and silver was absent also from the bone marrow and megakaryocytes. The fibrous tissue incorporated in the porous shafts was free from silver.

The most diffuse deposits of silver were in the chondro-osteoid, immediately adjacent to the cartilage. This tissue contained many included cartilage cells, and the silver was associated with these cells and with the osteoblasts surrounding the trabeculae, leaving only small amounts of intervening bone matrix unaffected. In the control sections this tissue was completely free from silver.

In the sections from the puppies in groups I and II the distribution of phosphatase was largely obscured by the calcified matrix of the cartilage and bone. In the animals from these groups, however, it was also seen that osteoid borders on calcified trabeculae were free from the silver stain in the incubated, as well as in the control, sections (fig. 7). In the incubated sections the osteoid borders appeared as thin strips (about 5 microns) of pink-staining matrix between the heavy deposits of silver in the calcified bone and in the osteoblastic layers on the surface of the trabeculae (fig. 7 *B*).

12. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42**:23. 1939.

COMMENT

The preparation of a diet deficient in phosphorus but otherwise adequate has recently been considered by several authors. The diet used in the present study contained the protein suggested by Schneider and Steenbock [2] and a low phosphorus liver extract as the source of some members of the vitamin B complex recommended by Jones.[1] This diet is not considered ideal or even adequate for an indefinite period. That a diet may permit good growth for a considerable period and still be inadequate was indicated by the results of McKibbin, Black and Elvehjem.[13] The present diet was convenient to prepare and was adaptable for the study of either calcium or phosphorus deficiency by simply altering the salt mixture. Under the conditions of this experiment the difference between the control and low phosphorus groups must be ascribed to the deficiency of phosphorus or to some secondary effect arising from this deficiency.

The decrease in the concentration of inorganic phosphate in the serum and whole blood of the animals receiving the low phosphorus diet was as anticipated. The lack of influence of vitamin D on the serum phosphate at the very low levels of phosphorus intake maintained in this experiment was definite. The decrease in organic acid-soluble phosphorus reported by Kay [14] and by Guest and Rapoport [15] to occur in rats with low phosphorus rickets was absent or minimal. This experiment thus failed to demonstrate an equilibrium between the organic acid-soluble phosphorus and the inorganic phosphorus of the blood.

There was a striking difference in the serum calcium curves of the puppies receiving the low phosphorus diet and the control puppies. All of these animals were offered the same amounts of calcium, but the intake of those in groups III and IV, in which hypercalcemia occurred, was actually less than that of the puppies in the control groups. Although a rough correlation between the intake of calcium and its concentration in the serum (Shohl and Wolbach [16]) of rats receiving diets deficient in phosphate has been observed, the occurrence of hypercalcemia of this degree and duration in experimental rickets does not appear to have been recorded.

We cannot account adequately either for the rise in serum calcium or for its subsequent fall. The well known inverse ratio between calcium and phosphorus in the serum operates to prevent a coincident rise in

13. McKibbin, J. M.; Black, S., and Elvehjem, C. A.: Am. J. Physiol. **130**: 365, 1940.

14. Kay, H. D.: J. Biol. Chem. **99**:85, 1932.

15. Guest, G. M., and Rapoport, S.: Am. J. Dis. Child. **58**:1072, 1939.

16. Shohl, A. T., and Wolbach, S. B.: J. Nutrition **11**:275, 1936.

both (McLean and Hinrichs [17]); it does not ordinarily lead to a rise in either when the other is depressed. On the other hand, a fall in serum phosphate would *permit* a rise in serum calcium if other and conducive factors were present. The fall in the serum calcium after the third bleeding followed a reduction in the mineral content of the basic diet to one-half the previous levels. There were increasing debility and inanitition at this time, and the influence of time itself, permitting an adjustment of the animals' regulatory mechanisms, cannot be excluded. It would be important to know whether the parathyroid glands were in a state of functional hyperactivity at the peak of the serum calcium curves, indicating that the hypercalcemia was the result of such hyperactivity, or whether the glands were in a state of hypoactivity secondary to alimentary hypercalcemia. This problem is being further investigated.

No evidence of healing in the animals receiving vitamin D was observed in roentgenograms of the radius and ulna or in histologic preparations of these bones and of the costochondral junctions. This is contrary to results observed in rats by Schneider and Steenbock,[2] but similar to those observed by Follis, Day and McCollum.[18] The dose of vitamin D used in the present study (50 U. S. P. units per kilogram per day) was low but adequate for the protection of puppies with an adequate mineral intake.

The histologic pictures of the bones in groups III and IV are identical. They are characteristic of florid rickets and except for the positive and unequivocal differentiation between calcified and uncalcified tissues (fig. 5) add nothing to the pathologic anatomy of this condition. The observations in groups I and II, however, are of considerable interest. The bones from group I when examined in undecalcified sections impregnated with silver nitrate were those characteristic of normal animals as described by McLean and Bloom.[10] The bones from group II, while apparently identical with those from group I when examined in decalcified sections, were seen in undecalcified sections to exhibit a marked increase in osteoid tissue in the secondary substantia spongiosa and shafts of the ribs. The calcification of the primary substantia spongiosa and of the zone of provisional calcification in the cartilage remained normal. According to our interpretation, these findings represent a form of minimal or borderline rickets, ordinarily not recognizable by other means. This condition was obviously associated with deprivation of vitamin D and developed in spite of an adequate mineral intake.

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17. McLean, F. C., and Hinrichs, M. A.: Am. J. Physiol. **121**:580, 1938.

18. Follis, R. H., Jr.; Day, H. G., and McCollum, E. V.: J. Nutrition **20**:181. 1940.

Table 3 illustrates the correlation between the state of calcification in the bones and the concentrations of calcium and phosphate in the serum. For groups I and II calculations were made only for the fifth and sixth (final) bleedings, as being most representative of the conditions at autopsy. For groups III and IV calculations were made for the first or control bleeding, for the third bleeding, representing the peak of the serum calcium curve, and for the sixth, or final bleeding, representing the conditions at autopsy. Calculations were made of the

TABLE 3.—*Correlation Between State of Calcification of the Bones and the Ion Products* $[Ca^{++}] \times [HPO_4^{=}]$ *and* $[Ca^{++}]^3 \times [PO_4^{=}]^2$ *in the Serum* *

Group	Animal	Bleeding	Total Ca, mM. per Liter	Total P, mM. per Liter	Ca^{++}, mM. per Kg. H_2O	$HPO_4^{=}$, mM. per Kg. H_2O	$PO_4^{=}$, mM. per Kg. H_2O	$[Ca^{++}]^3 \times [PO_4^{=}]^2$ — log	$[Ca^{++}] \times [HPO_4^{=}]$ — log	Calcification of New Bone
I	A_1	5	3.25	2.77	1.70	2.48	10.6	**22.2**	**5.37**	++
		6	3.00	2.61	1.55	2.34	10.0	**22.4**	**5.44**	
	A_5	5	3.27	2.85	1.70	2.56	10.9	**22.2**	**5.36**	++
		6	2.90	2.31	1.49	2.08	8.9	**22.8**	**5.51**	
II	B_{10}	5	3.20	2.32	1.67	2.08	8.9	**22.4**	**5.43**	±
		6	2.95	2.02	1.51	1.81	7.7	**22.7**	**5.56**	
	C_{13}	5	3.02	2.48	1.57	2.22	9.5	**22.5**	**5.43**	±
		6	2.85	2.16	1.48	1.94	8.3	**22.8**	**5.54**	
III	B_{11}	1	2.80	2.94	1.43	2.63	11.2	**22.4**	**5.43**	0
		3	4.30	0.93	2.22	0.83	3.6	**22.7**	5.71	
		6	3.12	0.81	1.60	0.73	3.1	23.4	5.94	
	C_{17}	1	2.90	3.23	1.49	2.90	12.3	**22.3**	**5.37**	0
		3	4.40	1.16	2.40	1.05	4.4	**22.6**	5.60	
		6	3.22	0.76	1.68	0.68	2.9	23.4	5.94	
IV	A_3	1	2.95	2.61	1.51	2.34	10.0	**22.5**	**5.45**	0
		3	4.32	0.72	2.35	0.64	2.7	**23.0**	5.82	
		6	3.02	0.63	1.56	0.57	2.4	23.7	6.05	
	C_{12}	1	2.95	2.85	1.51	2.56	10.9	**22.4**	**5.41**	0
		3	3.80	0.90	2.02	0.81	3.4	**23.0**	5.79	
		6	2.80	0.96	1.43	0.94	3.6	23.4	5.91	

* Ion products are expressed as negative logarithms. Calculations of $HPO_4^{=}$ and $PO_4^{=}$ were made by use of dissociation constants of H_3PO_4 from Sendroy and Hastings.[21] A *p*H value of 7.4 and total protein of 6.2 Gm. per liter are assumed. Ion products indicating saturation or supersaturation are in bold face type.

ion products $[Ca^{++}] \times [HPO_4^{=}]$ and $[Ca^{++}]^3 \times [PO_4^{=}]^2$, both being expressed in table 3 by their negative logarithms.

Shear, Washburn and Kramer [19] found $pK'_{s.p.}$ $CaHPO_4 = 5.47 \pm 0.01$. Consequently, any ion product the negative logarithm of which is numerically lower than 5.47 indicates saturation or supersaturation with secondary calcium phosphate, while values numerically higher than 5.47 indicate undersaturation. Logan and Taylor [20] found $pK'_{s.p.}$

19. Shear, M. J.; Washburn, M., and Kramer, B.: J. Biol. Chem. **83**:697, 1929.
20. Logan, M. A., and Taylor, H. L.: J. Biol. Chem. **119**:293, 1937.

$Ca_3(PO_4)_2 = 23.1 \pm 0.4$. The degree of precision attaching to this value is not so great as in the case of secondary calcium phosphate, but if it is accepted as valid, ion products with negative logarithms numerically lower than 23.1 indicate supersaturation with tertiary calcium phosphate, while values above 23.1 indicate undersaturation. All calculations of $[HPO_4^{=}]$ and $[PO_4^{\equiv}]$ are made by use of the dissociation constants of H_3PO_4 as given by Sendroy and Hastings.[21] It should be noted that at p_H 7.4, the hydrogen ion concentration assumed in making these calculations, the relative concentrations of these ions are such that the solubility product of $CaHPO_4$ can be reached only in a solution already supersaturated with $Ca_3(PO_4)_2$. Consequently, serum may be supersaturated with $Ca_3(PO_4)_2$ and undersaturated with $CaHPO_4$, but the reverse is not possible.

From table 3 it will be seen that all the serums examined were supersaturated with $Ca_3(PO_4)_2$, except for the final bleedings in the 4 rachitic animals in groups III and IV. In these the ion products indicate undersaturation. Obviously, then, no correlation could be found between these ion products, the state of calcification in the bones and the solubility product for $Ca_3(PO_4)_2$.

The initial bleedings from the puppies in groups III and IV all showed saturation with $CaHPO_4$. The marked rise in serum calcium in the puppies in these groups was not sufficient to maintain saturation in the presence of diminishing concentrations of phosphate, and at the time of the third bleeding, at the peak of the serum calcium curves (fig. 1), all of the serums were clearly undersaturated. The condition of undersaturation with $CaHPO_4$ was even more striking at the time of killing the animals, when the serum calcium values had returned to normal levels. There was thus, in groups III and IV, a correlation between the ion product $[Ca^{++}] \times [HPO_4^{=}]$, the state of calcification in the bones of the animals and the solubility product for $CaHPO_4$.

From four bleedings of puppies in group I only one value for the negative logarithm of the ion product was higher than 5.47, and the negative logarithm of the average of the four ion products was 5.42, indicating that these serums were saturated with $CaHPO_4$. From corresponding bleedings of group II the negative logarithm of the average was 5.50 and for two of the four products was above 5.47, both of them being from the final bleedings. We should not attach too much weight to the differences between the ion products in groups I and II, because of the small numbers of animals involved, but it will be noted

21. Sendroy, J., Jr., and Hastings, A. B.: J. Biol. Chem. **71**:783, 1927.

that the puppies in group I had normally calcified bone (fig. 3), while the animals in group II had an excessive amount of uncalcified osteoid tissue (fig. 4), diagnosed as borderline rickets. That the ion products were in one instance above and in the other below the solubility products given by Shear, Washburn and Kramer [19] is of great interest and adds weight to their conclusion that this ion product is critical for calcification.

The observations with respect to phosphatase were: increase in serum phosphatase in groups III and IV; variation in the phosphatase content of the cortex of the kidney; localization of phosphatase in relation to cartilage cells and osteoblasts. While the significance of these findings is still obscure, owing to the uncertainties as to the origin of bone phosphatase and its relationship to the calcification of bone, the observation that osteoid tissue in both normal and rachitic animals is devoid of phosphatase is of great interest. This tissue was ready to calcify (McLean and Bloom [10]) in the presence of suitable humoral conditions. That the phosphatase is associated with the osteoblasts rather than with the calcifiable tissue itself may be assumed to have bearing on the problem of the role of phosphatase in calcification.

SUMMARY

A diet is described which is suitable for the study of virtually complete phosphorus deficiency in dogs. When this diet was fed to puppies, florid rickets (observed roentgenologically and confirmed histologically) resulted, regardless of whether or not vitamin D was given in amounts sufficient to protect puppies with an adequate mineral intake.

Marked hypercalcemia, possibly of alimentary origin, developed in all the animals fed this diet. It reached its maximum on the thirty-second day of the experimental diet. The intake of calcium was then reduced, and the serum calcium concentrations decreased and were at normal levels when the experiment was terminated on the hundredth day. The hypercalcemia was somewhat more marked in the puppies receiving vitamin D. There was a striking decrease in the inorganic phosphorus concentrations in the serum and whole blood of the animals receiving the diet; this diminution was unaffected by vitamin D. The acid-soluble organic phosphorus was not significantly altered.

Puppies on the control diet, identical with the low phosphorus diet except for adequate amounts of phosphorus and vitamin D, grew normally with normal calcification of the bones. Puppies on the control diet but without vitamin D grew normally but showed increased amounts of uncalcified osteoid tissue in the spongy bone and shafts of the ribs, diagnosed as borderline rickets. This was correlated with slightly lower

concentrations of phosphate in the serum and with ion products $[Ca^{++}] \times [HPO_4^{=}]$ which indicated slight undersaturation of the serum with $CaHPO_4$.

The serum phosphatase activity was increased in the rachitic animals, and there was a moderate increase in the phosphatase content of the costochondral junctions of these animals and of the control animals deprived of vitamin D. The phosphatase in the bones was demonstrated to be associated with hypertrophic cartilage cells and with osteoblasts. It was not found in uncalcified osteoid tissue of either normal or rachitic animals.

INSUFFICIENCY OF THE AORTIC VALVE DUE TO SYPHILIS

A STUDY OF ITS GENESIS

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Gross and microscopic studies of aortic valves that have been rendered incompetent by syphilis have demonstrated that the primary lesions are in the aorta, whence they spread to the aortic ring and valve.[1] The current belief is that the types of distortions produced resulting in insufficiency are those recorded by Bell[2] as "(a) separation of the valves at the commissures; (b) thickening and retraction of the leaflets; (c) stretching of the aortic ring."

Separation of the valve cusps at the commissures has been attributed to: a wedge-shaped proliferative lesion in the aortic wall which separates the cusps[3]; sclerosis and shrinkage of valve tissue,[4] and adhesions which form between the lateral margins of the leaflets and the adjacent aortic wall.[5]

Thickening of the leaflets is limited almost exclusively to sclerosis of a cordlike type along their free margins; the involved tissue shrinks and prevents the cusps from meeting along their entire normal line of closure in diastole.[6] These changes are said to be due to the spread of the syphilitic inflammation from the aortic wall through the commissures and along the free margins or, rarely, from the bottom of the sinuses of Valsalva through the bodies of the cusps.[7]

"Retraction" as applied to aortic cusps affected by syphilis has been used with varying shades of meaning. Because of the lack of a generally accepted definition, aid was sought from twelve eminent pathologists.[8]

From the Department of Pathology, College of Medicine, Howard University.

1. Willius, F. A.: Proc. Staff Meet., Mayo Clin. **12**:605, 1937.

2. Bell, E. T.: A Text-Book of Pathology, ed. 3, Philadelphia, Lea & Febiger, 1938, p. 516.

3. Martland, H. S.: Am. Heart J. **6**:1, 1930.

4. Miloslavich, E. L.: Arch. Dermat. & Syph. **12**:41, 1925.

5. Saphir, O., and Scott, R. W.: Am. J. Path. **3**:527, 1927; Tr. A. Am. Physicians **42**:36, 1927; Am. Heart J. **6**:56, 1930.

6. Lupu, N.: Schweiz. med. Wchnschr. **50**:915 and 940, 1920.

7. Benedict, J.: Virchows Arch. f. path. Anat. **281**:780, 1931.

8. Personal communications to the author from eleven of twelve well known pathologists from whom a definition of the term "retraction" was requested.

Eight of the eleven who answered defined "retraction" as meaning shortening of the cusp from the free border to the base; one believed that it might mean shortening both from the free border to the base and from commissure to commissure; one believed that it means "thickening and shortening of the valves and also separation at the commissures," and one did not favor the use of the term. It would appear, therefore, that most pathologists are agreed that "retraction" means shortening of the cusps from the free border to the base, but relatively little effort has been made to explain how this shortening is effected.

Dilatation of the aortic ring is believed to have its origin, like aneurysms, in the destructive lesions produced by syphilis and the varying intra-aortic pressure. Stretching of the aortic cusps due to dilatation of the ring has been reported as the cause of the valvular deformities found in syphilis.[9]

Up to the time when this study was undertaken, there were available two reports which did not fit in with the usually accepted opinions. Scott,[10] in 1924, described a curious sagging of the free border of the aortic cusps, which he attributed to a nodular plaque at the commissure which, by gradual elevation or extension downward, carried with it the valve insertions. In later reports with Saphir,[5] Scott appears to have abandoned this earlier opinion. Mallory,[11] in 1929, described the valvular lesions characteristic of syphilitic aortitis and accounted for them by stating that "there is more or less fibrosis and thickening of the cusps close to the point where they reach the aorta, but the process is primarily destructive." In an accompanying sketch he portrayed the genesis of the valve deformities in a manner identical with that described recently by Norris [12] and with the impressions I have held independently for a number of years. Because their impressions and mine are not enjoying wide acceptance today and because these impressions appear to represent a sound evaluation and interpretation of the deformities of the aortic valve due to syphilis, it seemed desirable to record them anew, giving reasons and additional evidence in their support.

MATERIALS AND METHODS

Of 692 necropsies performed at Freedmen's Hospital on subjects over 20 years of age from Oct. 1, 1931 to Nov. 1, 1940, 21 disclosed gross alterations of the aortic ring and valve sufficient to confirm the clinical diagnosis of syphilitic aortitis with aortic insufficiency. In 6 additional cases with sufficient anatomic

9. Krischner, H.: Virchows Arch. f. path. Anat. **282**:30, 1931.
10. Scott, R. W.: Arch. Int. Med. **34**:645, 1924.
11. Cabot Case 15481, New England J. Med. **201**:1108, 1929.
12. Norris, J. C.: South. M. J. **32**:475, 1939.

justification for such a diagnosis the condition was not identified clinically. Nearly 300 nonsyphlitic hearts were available for comparison with this syphilitic material.

In the gross study of the syphilitic hearts, special attention was directed to (*a*) the shape and structure of each cusp, (*b*) the positions of the commissural extremities of the two cusps at any one commissure, (*c*) the relative positions of the three commissures, (*d*) the relationship of retraction to separation of the cusps at the commissures, (*e*) the presence or absence of a wedge-shaped proliferative lesion in the aortic wall associated with the separation of cusps at a commissure, (*f*) the presence or absence of adhesions within the sinuses of Valsalva between any part of the valve and the aortic wall, (*g*) the presence or absence of demonstrable dilatation of the aortic ring and (*h*) the relative amount of sclerosis as against destruction or loss of cusp structure.

Several specimens were selected, showing sclerosis of the cusps with separation at the commissures, retraction and other syphilitic deformities, and an attempt was made to reproduce them by dissection of normal aortic valves. Obviously, sclerosis could not be reproduced in this way.

Tissue blocks were taken from the commissural regions of selected syphilitic and nonsyphilitic hearts, and from these sections were made and stained by the hematoxylin-eosin and the combined Van Gieson and elastic tissue methods, in order to determine the character and extent of the syphilitic process at these sites. This paper, however, is concerned primarily with the gross observations.

OBSERVATIONS

The distortions of the aortic valves observed in the syphilitic material included at one extreme chronic lesions with striking proliferative changes. These specimens showed sclerosis with the well known "curling" and shortening of the free edges of the cusps, separation of the cusps at the commissures and retraction. At the other extreme were specimens of relatively acute lesions, consisting in the main of destruction of valve substance at the commissures. In between were gradations from one extreme to the other, and in this intermediate group when special attention was directed to the eight features listed in a foregoing paragraph, the character of the valvular lesion found in each instance, i. e. feature "h," was observed to affect most of the other seven.

There are on record accurate descriptions of both the chronic [13] and the acute [12] types of syphilitic valvular lesions, so that a repetition here is not necessary. The distortions found in the intermediate group which are significant for the purposes of this paper include: (*a*) one or more cusps which, though not relaxed or elongated, can be folded back into the ventricle (fig. 1 *A*); (*b*) one or more cusps presenting a thickened free border which is curved or slightly angulated so that the central part lies above a straight line drawn from one commissural attachment of the cusp to the other (fig. 2 *A*); (*c*) inequality of attachment of the

13. Clawson, R. J., and Bell, E. T.: Arch. Path. **4**:922, 1927.

two cusps at the same commissure so that one lies at a lower level than the other (figs. 1 *A* and 2 *A*); (*d*) the three commissures out of normal alinement so that one or more lie below the normal position, leaving

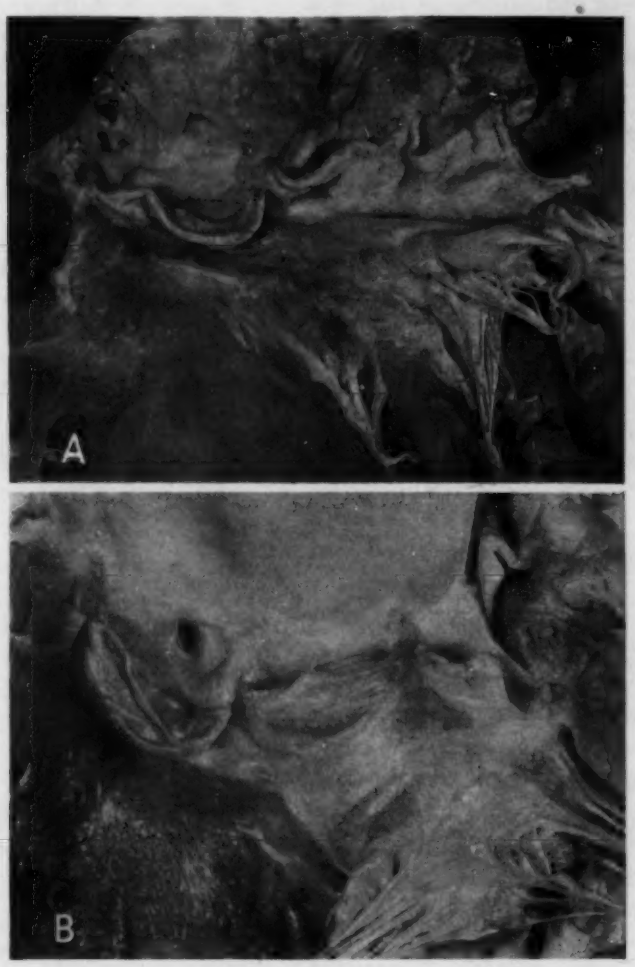

Fig. 1.—*A*, syphilitic aorta and aortic valve showing (1) a cusp folded back into the ventricle owing to gradual dislodgment from the aortic wall at a commissure where the adjacent cusp is not affected similarly, (2) scarring of the aorta and inequality of cusp attachments at this commissure, (3) a curved free border of the adjacent cusp, with convexity directed away from the ventricle, (4) thickened free borders of all cusps and (5) slight separation of the cusps at another commissure. The process in this specimen is more fulminating than in many a "typical" case. *B*, aortic valve of a nonsyphilitic normal heart, altered by dissection to reproduce the distortions due to syphilis in *A*. An impression of scarring is given by the defects left where the cusps were dissected away from the aortic wall, and the apparent thickening of their borders is due to folding of the loosened parts of the cusps into their respective sinuses of Valsalva.

behind a scarred intima (figs. 1 to 3); (*e*) the presence of retraction when separation of the cusps at the commissures is apparent, and absence or a mild degree of retraction when there is no separation of the cusps at the commissures.

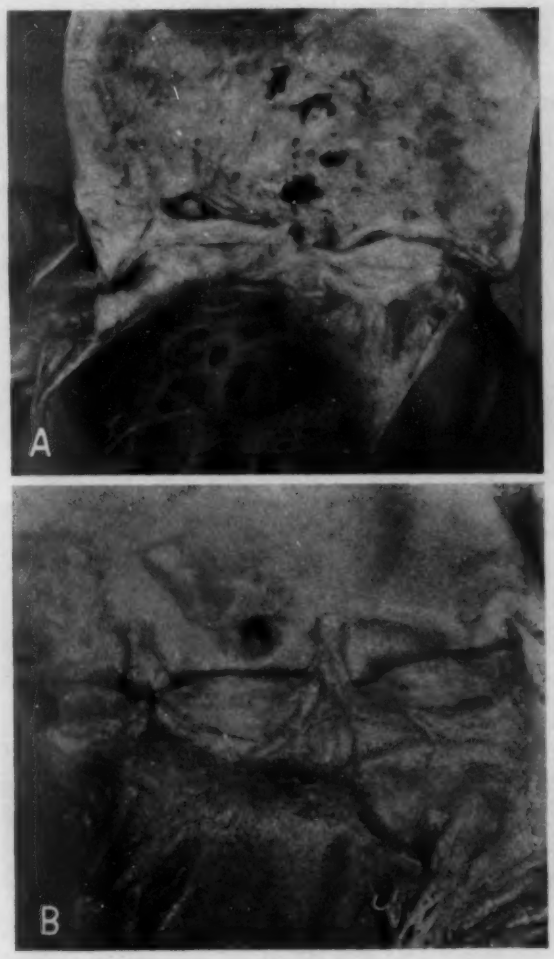

Fig. 2.—*A*, an example of chronic syphilis of the aortic valve showing separation of the cusps at the commissures and thickening and retraction of the leaflets. It shows also inequality of cusp attachments at the same commissure and two cusps whose angulated or curved free borders have their convexity directed away from the ventricle. *B*, a normal aortic valve altered by dissection to reproduce the syphilitic distortions in *A*. Notice that the dissection has caused the three commissures to be out of alinement.

Separation of the cusps at the commissures was found commonly without a local proliferative wedge-shaped lesion, and in the presence

of such a lesion there was little to suggest that the cusps had been pushed apart by it. Further, there were no specimens with demonstrable adhesions between cusps and the adjacent aortic wall above or within the sinuses of Valsalva.

The most impressive examples of the destructive type of lesion include a heart with a fibrous loop near the center of the right anterior cusp (fig. 3 *A*) and a specimen showing an apparent fusion between the

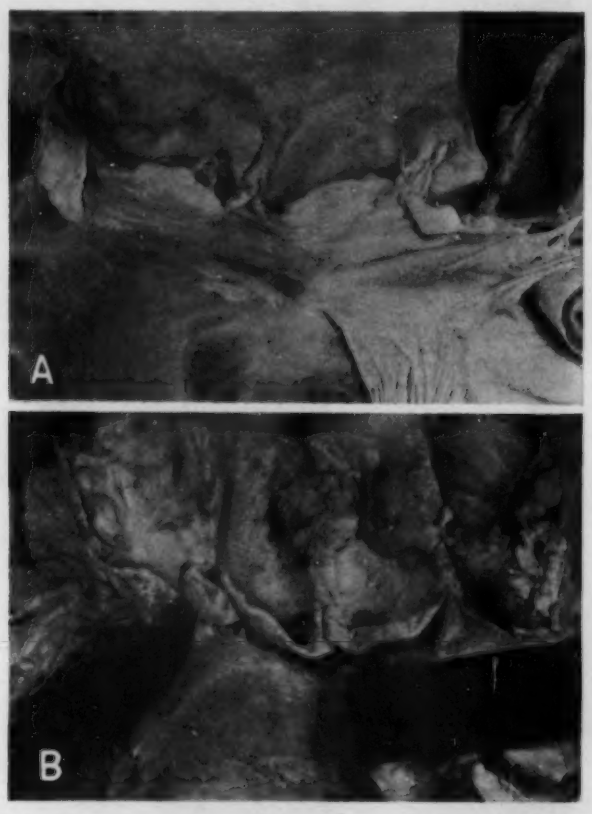

Fig. 3.—*A*, syphilitic destruction of cusp substance at one commissure, allowing one cusp to retain as a fibrous loop one of the fenestrations commonly found at this site, while the other cusp retained only a small fibrous nodule. In spite of the destruction of valve substance, there is no separation of the cusps at this commissure. *B*, a destructive syphilitic lesion allowing two cusps to remain attached to each other while being dislodged from their attachment to the aortic wall at their commissure.

right anterior and posterior cusps (B. N. A. nomenclature) in the absence of either Mönckeberg's sclerosis or evidence of preexisting rheumatic or bacterial valvulitis (fig. 3 *B*).

In this series, dilatation of the aortic ring appears to have been a significant factor in the production of insufficiency, for 20 of the 27 hearts had aortic rings measuring over 7 cm. in circumference, and in 11 the rings were over 8 cm. in circumference.

The attempt to reproduce by dissection of normal valves the valvular distortions due to syphilis (excluding sclerosis and healing) resulted in rather faithful reproductions of the distortions herein considered to be characteristic. It was possible by dissection to so deform the valves as to cause the cusps to fold back into the ventricle; to present an angular or curved free border with convexity away from the heart; to cause the valve commissures to be out of alinement; to cause inequalities in the point of attachment of the cusps at one commissure, and to produce a picture akin to retraction, including separation of the cusps at their commissures (figs. 1 *B* and 2 *B*). Even apparent fusion of two cusps altered by retraction can be reproduced if care is taken to dissect through the commissure, separating both cusps from the aortic wall but not from each other and folding or trimming their free edges.

COMMENT

The varied deformities of aortic valves due to syphilis found in the material studied, ranging from valves with marked sclerosis to those with marked destructive features, appear to have in common two underlying processes: (*a*) a destructive inflammation of the aortic wall with resulting destruction and dislodgment of the cusp attachments at the commissures and (*b*) a reparative fibrosis. Varying proportions of these two processes seem to account adequately for the valvular distortions exemplified by different cases.

Reparative fibrosis or scarring is generally considered to be a primary factor in producing the classic syphilitic deformities of the aortic valve, but destruction of valve tissues by this process has been considered of little or no importance. It is readily admitted that fulminating syphilis may cause marked destruction of one or both cusps at a commissure [12] in addition to its well known devastating effects on the media of the aorta. Moreover, microscopic studies by Saphir and Scott,[5] Gross and Silverman [14] and Wilens,[15] as well as my own, have revealed that syphilis destroys tissue in the aortic wall at the commissures, with more or less sclerosis resulting. If it is granted as proved, therefore, that syphilis produces destructive lesions in the wall of the aorta and at the commissures of the aortic valve in all cases showing involvement of the valve and destruction of the very cusps themselves at the commissures when it is of the fulminating type, it seems logical to expect this destruc-

14. Gross, L., and Silverman, G.: Am. J. Path. **13**:389, 1937.

15. Wilens, S. L.: Arch. Path. **29**:200, 1940.

tive quality of the syphilitic inflammation to play a more or less conspicuous role in causing all aortic valve distortions which result in insufficiency. The validity of this interpretation becomes more and more apparent when consideration is given separately to the genesis of each of the various types of valve deformities listed here, in addition to the classic ones described by Bell.

Distortion of an aortic cusp such that a part of it folds or flaps back into the ventricle (fig. 1 *A*) can result either from stretching of the cusp along its free border or from a destructive lesion at one or both commissures. It does not appear likely that the first plays a part in syphilis, since fibrosis occurs with shortening and not stretching along the free margin of the cusps. Furthermore, if stretching of the cusps occurs when there is primary dilatation of the aortic ring, this is a compensatory process which does not allow an excess in length of the free margin. If, then, stretching does not account adequately for the distortion which allows one or more cusps to fold back into the ventricle, the second possibility, i. e., destruction of tissue, is perhaps the better explanation. This explanation appears sound not only for this type of distortion but also for the others, with one or more of which it is invariably associated.

The free borders of normal aortic cusps lie in a plane with their commissural attachments or display a slight curve with the convexity directed toward the ventricle. In syphilis this may be altered so that there is an angulation or curve with convexity directed away from the ventricle (fig. 2 *A*). This alteration can result either from proliferation of the central part of the cusp or from destruction at one or both commissural extremities. In syphilis the proliferation along the free border of a cusp is not limited to and is not more marked in the central part of the cusp. Moreover, since this alteration occurs in inverse proportion to the amount of sclerosis of the free border, destruction of valve substance at the commissure appears to be the more satisfactory explanation for its presence.

Wilens [15] has shown that in different subjects and indeed in the same subject there are variations in the amount of elastic tissue in the aortic media at the valve commissures. In syphilitic subjects he has found a significant direct correlation between the amount of elastic tissue originally available at the commissure and the presence there of lesions. Though this anatomic observation may serve as an explanation for the subject incidence and the commissural incidence of valvular defects in syphilis, it does not explain the frequent finding of an inequality of attachment of the two cusps at the same commissure. If, however, one considers that the destructive lesion need not be limited to the medial coat of the aorta at this site but may spread to the adjacent parts of the cusps, destroying these with varying degrees of intensity and rapidity.

one has an explanation for this finding. That this occurs is apparent in the more acute lesions to which reference has been made. The explanation just given seems adequate also to account for the frequent finding of a disturbance of the normal alinement of the three commissures so that one or more lie below the normal position.

The genesis of retraction does not appear to have been stated clearly or convincingly. The obvious explanation of shrinkage cannot be applied, since in syphilis the sclerosis is usually along the free border of the cusps and could not possibly result in shortening from the free border to the base. The terms "curling" and "rolled edge" suggest that the cusp edge becomes curled or rolled on itself. If this were the explanation for retraction, the affected cusps should retain their normal commissural positions, while becoming shortened from the free border to the base only at the central part. This, as a matter of fact, does not occur, and the microscopic study of such valves fails to support the idea of "curling." "Absorption" of the free border has been suggested,[8] and it appears to me to impart the correct idea, but so far as I know, this explanation has not been reported and it has been offered without the observation that the "absorption" (destruction, if you will) takes place primarily at the commissural ends of the cusps. It appears, therefore, from this review of possibilities, that retraction can be accounted for best on the basis of destruction of valve tissues and reparative fibrosis. It is to be noted that, because of the semilunar shape of the aortic cusps, any destruction of valve tissues at the commissures, beginning at the free border, must eventually lead to separation of the cusp remnants and, conversely, any appreciable separation must be due to destruction of valve tissues if there is associated retraction of the cusps.

Conceivably, a proliferative lesion at an aortic valve commissure might result in separation of the cusps from each other. It is unlikely, however, that such a fine localization would occur in syphilis with enough frequency to enable one to consider it as among the usual causes of separation of the cusps at a commissure. A proliferative lesion was found occasionally in the material studied, forming a hillock elevating one or more valve commissures, but it did not appear to cause separation of the cusps and was not associated with separation of the cusps unless there was evidence of destruction of valve tissues or of dilatation of the ring as well.

In the material studied, adhesions between the valve cusps and the adjacent aortic wall within the sinuses of Valsalva did not play a part in the genesis of insufficiency, although it is conceivable that they may do so. An instance of apparent adhesion between two cusps was found (fig. 3*B*). On close inspection, however, it was observed that the

apparent fusion had resulted from a fulminating syphilitic process in which both cusps were torn from their commissural attachment to the aortic wall while retaining their intimate attachment to each other. In a few instances changes were found which might have been interpreted as resulting from adhesions. There were commissures, for example, in which there was slight separation of the cusps at the free borders, while from 1 to 3 mm. proximally the cusps were in intimate apposition to each other. In each instance in which this change obtained, there occurred also dilatation of the aortic ring, syphilitic involvement of the aortic wall above the commissures and some sclerosis of the cusp borders at the commissures. These changes suggested that the defect had resulted from the effect of stretching of the ring at the commissures, thereby allowing the slight separation of the cusps noted. The genesis of this type of deformity, in my estimation, differs in no essential way from that of the valve deformities which are discussed in foregoing paragraphs; in this one the destructive effect of the syphilitic process is primarily in the aortic wall and the yielding occurs, as in aneurysms, because of the dilating effect of the systolic thrusts of the heart; in the former, the destruction affects the cusps more severely, which yield in diastole, instead of catching and supporting the aortic column of blood. In both the quality of the defect which may be found is modified by the rapidity with which the destruction progresses and the degree to which reparative fibrosis occurs.

From the foregoing comment it appears that an appreciation of the dual quality of the syphilitic inflammation, including destruction of tissue and sclerosis, offers a satisfactory explanation for the genesis not only of the classic deformities of the aortic ring and valve described by Bell but also for the acute ones described by Norris and for the intermediate ones described in this paper. When the destruction occurs rapidly, the subjects have the badly torn cusps which form the bases for occasional reports; when the destruction is less rapid and there is appreciable reparative fibrosis, the free border of the cusps may take a curved or angulated shape with convexity directed away from the heart; when destruction is very slow and the reparative process has ample time to shorten and thicken the free margins of the cusps, to round off the destroyed parts and to keep them covered with endothelium, then the best examples of retraction, curling of the edge and commissural separation are produced.

Just why these explanations of the genesis of the lesions in question do not appear to have been advanced prior to 1929 and why they have not received wide acceptance are questions which I cannot answer. I believe, however, that in order to become aware of the significant part which destruction of tissue plays in the syphilitic distortions of the aortic

ring and valve, it is necessary to study a series of cases which includes gradations from the very chronic to the fulminating types of lesions. This is not always possible.

SUMMARY

The syphilitic distortions of the aortic valve and ring generally accepted as the causes of insufficiency and the usual explanations of their genesis are described and discussed briefly.

The study of a series of 27 syphilitic hearts including gradations from chronic to fulminating lesions disclosed aortic valve distortions falling outside of those commonly described, which could not be accounted for by the generally accepted explanations of pathogenesis.

All of the valve distortions studied appeared to have been due to two processes: (*a*) a destructive inflammation of the aortic wall resulting in destruction and dislodgment of the cusp attachments at the commissures and (*b*) a reparative fibrosis.

These two processes, destructive inflammation and reparative fibrosis, are set forth and discussed as the basic factors in the genesis of aortic insufficiency due to syphilis. Reference is made to earlier brief statements to this effect by Mallory and Norris.

Support for the idea that destruction of tissue plays a significant part in the production of syphilitic valvular distortions is found in the partial reproduction of such distortions by dissection of normal valves.

CHEMICAL STUDIES OF THE SUSCEPTIBILITY TO SPONTANEOUS CARCINOMA OF THE MAMMARY GLAND IN MICE

II. INDICATIONS FOR THE ANALYSIS OF A GENETIC FACTOR OF SUSCEPTIBILITY IN RELATION TO NONGENETIC INFLUENCES

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Recent data have shown that tolerance to the lethal effects of salicylaldehyde (diluted with olive oil) is determined in part by the body weight, the age and the "genetic constitution" of the individual mouse.[1] Evidence is increasing that the C_3H, A and JK strains of mice can be classified in the same sequence according to (*a*) tolerance of the males to the lethal effects of salicylaldehyde, (*b*) the shift of hemoglobin per unit of time and (*c*) the tendency for breeder female mice to give rise to, or not to give rise to, spontaneous tumors of the mammary gland. Thus the conclusion is gradually being formed that these three seemingly independent phenomena have something in common.

EXPERIMENT

To the data already published on the C_3H, A and JK strains, similar data on the CHI, CBA and I strains of inbred mice are now added. These data are presented in the accompanying chart and table. A brief note on the characteristics of these three new strains in relation to susceptibility to carcinoma of the mammary gland may serve to orient them to the better known C_3H, A and JK strains. The CHI and CBA strains are similar to each other in that the breeder females of each strain have an intermediate degree of susceptibility to cancer; the CHI is slightly more susceptible than is the CBA, as indicated by the age distribution of spontaneous tumors. The average incidence of spontaneous tumors of the mammary gland in breeder female mice of these four inbred strains is as follows: (1) for female mice of the C_3H strain, the age incidence is between eight and nine months, (2) for A strain female mice it is between 10 and 11 months, (3) for CHI mice, between 22 and 24 months and (4) for CBA mice, between 24 and 26 months. These age distributions of spontaneous tumors were obtained on mice kept on

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1. Strong, L. C.: Yale J. Biol. & Med. **12**:255, 1940.

a mixed diet.[2] On the other hand, I [3] have published data which indicate that the age distribution (and consequently the percental incidence) of spontaneous tumors in mice is altered by the length of time the mice have been on a specific commercial diet. No attempt was made, however, to investigate further the nature of the dietary contribution to the origin of cancer in mice. The breeder female mice of the I strain are very resistant to spontaneous tumors of any nature—their life span is somewhat restricted, however, owing to an adenomatous hyperplasia of the stomach, as described by Andervont and Stewart.[4]

In this experiment the mice used belonged to six inbred strains, representing three classes based on an intrinsic susceptibility to carcinoma of the mammary gland, as follows:

(A) High susceptibility—C_3H and A

(B) Intermediate susceptibility—CHI and CBA

(C) Low susceptibility or complete resistance—I and JK

Male mice aged between 101 and 200 days were used. All mice were given injections of 8 mg. of salicylaldehyde dissolved in 9 parts of olive oil. If mice did not die from this injection within twenty-four hours, they were given another injection of the same amount of the material on the following day. This procedure was followed for six days, at which time the experiment was terminated because of the possibility that local changes at the site of injection might interfere with the systemic reaction of death or survival. The number of minutes each mouse survived was also recorded and the figures will be presented at some future time. In general, the data show the same trend as those on percental mortality.

An inspection of the chart and the table indicates that these strains of mice may be classified in the sequence of C_3H, A, CHI, CBA, I and JK on the basis of their tolerance to the lethal effects of salicylaldehyde. The same strains can be classified in the same sequence when susceptibility to spontaneous carcinoma of the mammary gland in breeder female mice is measured.

COMMENT

The genetic factor or factors that partially underlie resistance or susceptibility to carcinoma of the mammary gland in mice are somewhat indicated by the establishment of many inbred strains in which the incidence of cancer occurs in definite ratios characteristic of those strains, respectively. This appears in the work of Loeb and Lathrop and others. This definite ratio of the incidence of cancer to that of absence of cancer within an inbred strain is further indicated by the fact that:

In inbred strains of mice having a high breast tumor ratio the progeny descended from non-breast cancerous mothers do not show a low breast tumor

2. This diet consisted of Quaker rolled oats, Baugh & Sons scrapmeat, Klim powdered whole milk and salt (Strong, L. C.: Brit. J. Exper. Path. **37**:60, 1936).

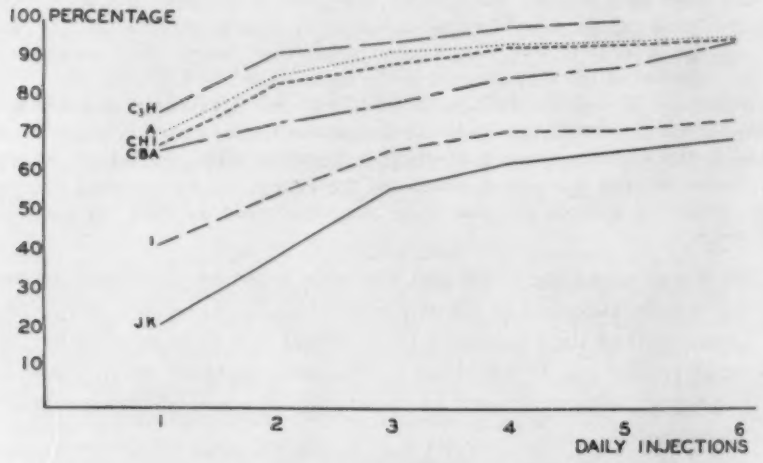
3. Strong, L. C.: J. Heredity **31**:9, 1940.

4. Andervont, H. B., and Stewart, H. L.: Science **86**:566, 1937. Stewart, H. L., and Andervont, H. B.: Arch. Path. **26**:1009, 1938.

ratio. Such mothers are able to transmit this type of neoplasm to their descendants although they themselves have not developed mammary cancer. In low-breast-cancer strains which have been inbred the progeny of breast cancerous mothers do not show a higher incidence than is characteristic of the stock.[5]

Data on the Mortality Following the Injection of 8 mg. of Salicylaldehyde Diluted with 9 Parts of Olive Oil into Male Mice of Six Inbred Strains

Strain	Total Number of Mice	Number and Percentage Dying in Six Successive Daily Injection Periods											
		1		2		3		4		5		6	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
C_3H	198	151	76.2	180	91.0	186	94.3	195	98.4	198	100	...	....
A	150	105	70.0	128	85.3	137	91.3	140	93.3	141	94.0	144	96.0
CHI	144	97	67.3	120	83.3	128	88.9	133	92.3	136	94.4	138	95.8
CBA	134	90	67.1	98	73.1	105	78.3	113	84.3	118	88.0	125	93.2
I	214	90	42.0	129	60.2	149	69.6	156	72.9	159	74.3	166	77.5
JK	162	36	22.2	63	38.8	92	56.7	102	62.9	108	65.6	112	69.1
Total	1,002												



A graphic presentation of the data on the cumulative percental mortality following injection of salicylaldehyde in oil as given in the table.

The genetic factor is further indicated by the occurrence of a definite ratio of resistant to susceptible mice in the F_1, F_2 and subsequent hybrid generations following the original hybridization cross of two individuals, one from a low cancer, the other from a high cancer, strain.

Strong has pointed out a probable genetic basis of susceptibility in emphasizing that Johannsen's principle of selection was ineffectual in changing the age distribution of spontaneous tumors of the mammary glands within an inbred strain. This suggests that the genetic susceptibility factor or factors for cancer are not unique but follow the same

5. Bittner, J. J.: Am. J. Cancer **39**:104, 1940.

biologic rule as do such genetic phenomena as size, growth and other similar quantitative processes within the organism.

The analysis of a genetic factor or factors for cancer susceptibility is complicated by several considerations as follows: (1) the ratio obtained in the backcross to the hypothetic recessive condition, which is not mathematically consistent with the ratio obtained in the F_2 generation; (2) a dietary agency in cancer, which exerts an influence possibly throughout the entire life span of the individual; (3) a maternal influence which, according to Little, is extrachromosomal (cytoplasmic?) but which is possibly the same agency that Bittner has called the "milk influence," received by a suitable (genetically determined?) individual through its milk supply from either its natural or its foster mother, and (4) a physiologic influence, indicated in (*a*) the forced suckling experiment of Bagg, (*b*) the degree of use to which the mammary gland has been subjected, e. g., by lactation, or (*c*) the hormonal influences, as demonstrated by the injection of estrogenic and other hormones. The last agency may overlap or even include the "physiologic use" agency. Since the induction of tumors of the mammary gland by estrogen is also strain limited, there is a possibility that this reaction or end result may have a genetic basis.

Hybridization experiments have indicated that a male mouse of a strain showing high susceptibility to carcinoma of the mammary gland is capable of transmitting this susceptibility to his daughters and females of subsequent generations, even though he himself or females of the immediate descent do not show cancer (unpublished data). This transmission, however, can be detected only when suitable correction for the "milk factor" of Bittner has been made. It has been shown by many investigators, following the lead of Lacassagne, that this intrinsic susceptibility to cancer of the mammary gland may be brought to fruition in a male mouse by the injection of estrogenic hormones. The role of the hormone would be, then, in the stimulation of an inert or rudimentary mammary gland, associated with the partial feminization of the male individual.

The present data indicate that tolerance to the lethal effects of salicylaldehyde may be a measure of cancer susceptibility (possibly a measure of the genetic, or intrinsic, factor). Below a certain threshold there is an indication of resistance to cancer; above that threshold susceptibility to cancer. The difference between cancer resistance and cancer susceptibility is greater than the difference between an intermediate and a high degree of susceptibility. This concept is further indicated in many experiments in which it is clear that the mechanism determining the actual onset of cancer is a very delicate one. Any deviation one way or the other is apt to influence it. Thus the new

data indicate the possibility that the difference between cancer resistance and cancer susceptibility is a quantitative not a qualitative difference, as was indicated in a previous publication.[2]

At the present time, therefore, there is evidence obtained by many investigators that in the origin of carcinoma of the mammary gland there are at least four agencies or sets of agencies, as follows: (1) a genetic susceptibility factor or factors, of which the tolerance to the lethal effects of salicylaldehyde may be in part a measure; (2) the physiologic state of the mammary gland as influenced by use and by hormones; (3) the "milk factor" of Bittner, and (4) a dietary agency exerting an influence over an extended period, perhaps the entire individual life span.

CONCLUSIONS

Tolerance to the lethal effects of salicylaldehyde parallels and may be used as a measure of the intrinsic susceptibility which partly determines carcinoma of the mammary gland in mice.

The genetic factor or factors may exert an influence on the individual prone to have carcinoma of the mammary gland at some future time apparently in two directions: (1) the development of the physiologic state of the organism of which tolerance to the lethal effects of salicylaldehyde may be an index and (2) the physiologic state of the mammary gland as influenced by hormones.

AN IMPROVED METHOD FOR EXPERIMENTAL GRAFTING OF SKIN

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The present report concerns a simple but effective method for transplanting skin in experimental animals—a method which gives from 80 to 90 per cent successful grafts. Numerous technics for this purpose have been used heretofore, but none has been particularly satisfactory, the incidence of "takes" being small even when autotransplants were made. The most successful results with autotransplants were those reported by Saxton, Schmeckebier and Kelley [1] and Sale,[2] who obtained 44 and 68 per cent "takes," respectively. These authors utilized the method of Loeb.[3] Failure of their grafts to grow seemed to be due either to dehydration of the graft or to trauma.

The method described here minimizes the dangers of trauma and dehydration. Essentially, it is a modification of the "skin flap technic" described by Carnot and Deflandre.[4]

EXPERIMENTAL METHODS

Preliminary study demonstrated the need for modifying the technic of Carnot and Deflandre. These investigators placed small grafts beneath very thin epidermal flaps about 1 cm. square. This procedure was not satisfactory, because of the difficulty of preparing epidermal flaps in thin-skinned animals and because such thin flaps tended to curl, leaving the underlying grafts exposed. These difficulties have been avoided by using a skin flap of both dermis and epidermis and at a later time removing the overlying skin so that the growth of the graft could be followed.

Briefly, the technic used was as follows: A semicircular flap of skin, extending down to the underlying connective tissue, was raised up, and a graft 1 mm. in diameter and similar to the small deep graft of Davis [5]

From the Institute for Medical Research, Christ Hospital.

1. Saxton, J. A.; Schmeckebier, M. M., and Kelley, R. W.: Biol. Bull. **71**: 453, 1936.
2. Sale, L.: Arch. f. Entwcklngsmechn. d. Organ. **37**:248, 1913.
3. Loeb, L.: Arch. f. Entwcklngsmechn. d. Organ. **6**:1, 1897.
4. Carnot, P., and Deflandre, C.: Compt. rend. Soc. de biol. **3**:178, 1896.
5. Davis, J. S.: Ann. Surg. **89**:902, 1929.

was placed in the defect, next to the base of the flap. The flap was lowered in place and left undisturbed for eighteen to twenty-one days. Then, with the animal under light ether anesthesia, the overlying tissue was removed with a scalpel. The exposed grafts were examined macroscopically at various intervals.

As a control procedure, the method of Loeb as modified by Saxton and co-workers was used. After this method, a similar graft 1 to 2 mm. in diameter was placed on a denuded area and covered with an alcohol-soaked cotton dressing, which was held in place with collodion.

All the graftings were autotransplantations performed on young guinea pigs weighing between 230 and 370 Gm. In all instances either black or brown skin was transplanted to white skin. The operative procedures were carried out with the animals under anesthesia induced with pentobarbital sodium. The preparation of the skin involved removal of the hair by clipping and shaving, washing of the skin with soap and water and final sterilization with tincture of merthiolate. Hemostasis was secured by pressure. Epinephrine was not used, since it seemed to induce curling of the graft.

Two series of experiments were performed. In the first, grafts were made indiscriminately on any part of the body. In the second, grafts were limited to the caudal area.

RESULTS

When the grafts were placed indiscriminately over the body, the modified Carnot-Deflandre technic gave results that were distinctly superior to those obtained with the Loeb method. One hundred and one grafts on 32 animals were prepared according to our technic, and 82 "takes" (81 per cent) were obtained. From the 53 grafts prepared by the Loeb procedure, only 27 "takes" (51 per cent) were obtained.

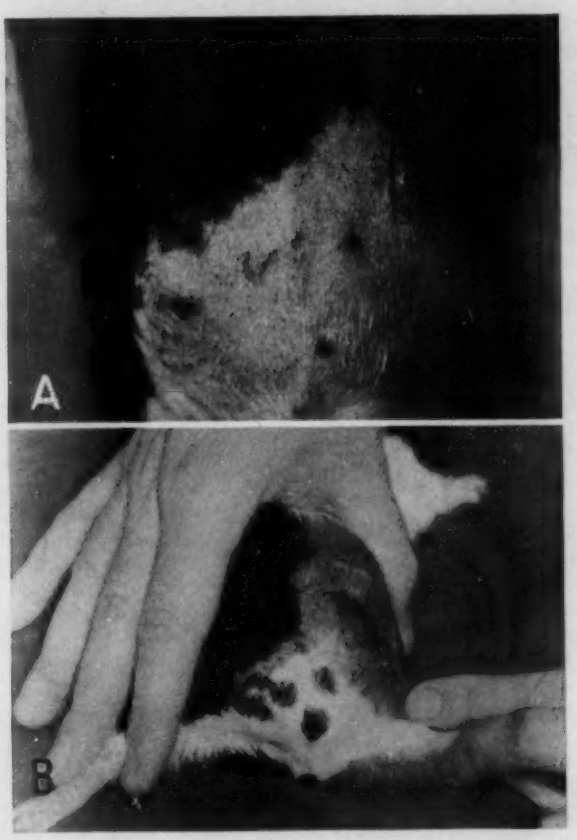
When the grafts were restricted to the caudal area, the success of the modified Carnot-Deflandre procedure was even greater. Twenty-six of 28 grafts (92 per cent) made with this method were successful, whereas only 12 "takes" were obtained from 28 Loeb grafts (42 per cent).

Actually, the skin flap technic was more successful than the foregoing figures indicate, for when the flaps were undisturbed for eighteen to twenty-one days, 100 per cent "takes" were obtained. In such successful grafts the function of the hair-producing cells was preserved and a rapid growth of either red or black hair proceeded soon after removal of the skin flap.

The number of successful grafts was reduced considerably when the skin flaps were disturbed prematurely, as by trauma. Under such cir-

cumstances the top layers of the grafts showed exfoliation. Only rarely did these grafts produce hair.

The grafts increased in size considerably during the period of observation; in 1 animal the surface area of the graft increased one hundred and fifty times. Figure 1 *A* and *B* shows typical growth of such grafts during two months' time. In *B* it is evident that the black pigment of



Growth of grafts during ninety days: *A*, grafts twenty-eight days after transplantation. *B*, same grafts ninety days after transplantation.

the graft has invaded the surrounding white skin. This finding is typical and agrees with that of Loeb and his co-workers but is contrary to the observations of Seevers and Spencer.[6]

In comparing the skin flap technic with the technics of Loeb and his co-workers, particularly with the Loeb method, the most obvious advan-

6. Seevers, C. H., and Spencer, D. A.: Am. Naturalist **66**:189, 1932.

tage is in the elimination of dehydration and trauma. By this improvement a graft is obtained which seems to be identical with normal skin.

SUMMARY

A successful method has been presented for grafting of skin on experimental animals. In brief, it consists of placing the graft beneath a full thickness flap of skin during the period of vascularization. At a later date the flap is removed. This method prevents early drying out of the graft and eliminates the necessity of dressings.

The best area for skin grafting by this technic is the dorsal caudal area of the guinea pig.

In most cases the hair follicles are preserved.

Case Reports

MONOCYTIC LEUKEMIA

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Although the occurrence of monocytic leukemia has been supported by abundant proof since Reschad and Schilling-Torgau [1] described the first case in 1913, doubt still lingers in the minds of certain investigators whether such a variety of leukemia exists at all. The evidence in favor of a "third" type of leukemia, however, has become almost incontrovertible. Rosenthal,[2] Dameshek,[3] Clough,[4] Osgood and Lyght,[5] Levine,[6] Doan and Wiseman,[7] Forkner,[8] Foord, Parsons and Butt,[9] Downey,[10] Doub and Hartman [11] and many more, referred to in many of the works just cited, have described an imposing number of cases that cannot be consistently placed in either the lymphoid or the myeloid group of leukemia. On combining clinical manifestations, hematologic characteristics and postmortem observations, a definite medical entity has apparently emerged from the uncertainty of earlier days. If the cases are studied by vital staining methods, as recommended by Sabin, Doan and Cunningham [12] and by MacKeith and Bailey,[13] and if the character of the oxidase granules is noted, the results leave little doubt. There is even a tendency to expand the grouping to take account of acute and chronic forms and other varieties, based on more refined cytologic differences. The most important group, perhaps, is based on cell origin. Downey [14] and Watkins and Hall [15] have discussed this under the head of two types, one that originates from the reticuloendothelial system,

From the Research Laboratories of the City of Chicago, Municipal Tuberculosis Sanitarium.

1. Reschad, H., and Schilling-Torgau, V.: München. med. Wchnschr. **60**:198, 1913.
2. Rosenthal, N.: M. Clin. North America **4**:1607, 1921.
3. Dameshek, W.: Arch. Int. Med. **46**:718, 1930.
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5. Osgood, C. W., and Lyght, C. E.: J. Lab. & Clin. Med. **18**:612, 1933.
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referred to as the Schilling type, and one that appears to come from myeloblasts, designated the Naegeli type. In the former there is hyperplasia of the reticular tissue with transitions from such cells by stages into monocytes but with no demonstrable "blast" cells present. This type may vary widely, according to Downey,[14] depending on the primitiveness of the particular cell of origin. In the Naegeli type, to which the case to be reported appears to belong, there is an apparent origin from a cell resembling the myeloblast with a proliferation of monocytoid cells that usually assume the varying stages of the monocyte. The degree of maturity of the cells varies considerably from case to case. Many cases are mistaken at first for instances of the myeloid type because of the presence of cells resembling, if they actually are not, myeloblasts, but the cell progeny becomes monocytoid cells and monocytes.

Occasionally cases have been reported in which the type cells follow a monocytic trend and then suddenly shift to a true myeloid type. Naegeli,[16] Levine [6] and Watkins and Hall [15] have reported this type of mixed cell. The reverse, i. e., the shift from the myeloid to the monocytic type, has also been reported by Craciuneanu and Calalb [17] and Watkins and Hall.[15]

The shifting from one type to the other, however, has been disputed by Forkner,[18] who is dubious of such "miracles," and Doan and Wiseman [7] are of the opinion that while there may be, and usually is, a shift to the left in all cells, there is only one strain that reveals *abnormal* cytologic changes. As will be pointed out later, the strongest argument for the unitarian point of view of blood origin, i. e., the prevalence of environmental over hereditary factors, could also support such a shift in cell characteristics, but some of the unitarians do not believe in the existence of monocytic leukemia.

Besides the hematologic and cytologic characteristics, Forkner [18] has also pointed out that the associated clinical phenomena may be peculiar to each type. In the monocytic form there are extreme enlargement of the spleen with little enlargement of the lymph nodes and not only bleeding but ulceration of the gums. The other two forms do not have this combination of symptoms, and the lymphatic type is separated by its tendency to form large lymph nodes. Doan and Wiseman [7] are less certain about the dependence on clinical difference because of a lack of enough statistical material up to the present time.

In order to add to the group of definite cases, 1 is now reported in detail.

REPORT OF A CASE

A white woman aged 25, of Italian descent, a nurse, was admitted to the Municipal Tuberculosis Sanitarium on Feb. 8, 1938. She knew of no definite contact with tuberculosis. The onset of her illness was influenzal. She had suffered from general malaise and weakness and had a temperature of 99.4 F. and hemoptysis of approximately 1 drachm (3.5 cc.) of blood. She was a well nourished

16. Naegeli, O.: Blutkrankheiten und Blut-Diagnostik, ed. 5, Berlin, Julius Springer, 1931.

17. Craciuneanu, A., and Calalb, G.: Sang **5**:397, 1931.

18. Forkner, C. E., in A Symposium on the Blood and Blood-Forming Organs, Madison, Wis., University of Wisconsin Press, 1939, p. 126.

TABLE 1.—*Results of Examinations of Blood*

Date	Hemoglobin (Per Cent)	Red Blood Cells (Millions)	White Blood Cells per Cubic Millimeter	Polymorphonuclear Leukocytes (Per Cent)					Monocytes				Lymphocytes (Per Cent)		Plasma Cells (Per Cent)
				Juveniles	Stab Forms	Segmented Forms	Eosinophils	Basophils	Group 1	Group 2	Group 3	Group 4	Large	Small	
3/20/40	60	3.00	3,900	1	10	18	0	0	3	40	1	6	14	7	0
3/22/40	60	3.59	3,950	0.5	7	20	0.5	0	2.5	45	0	4	11	9.5	0
3/23/40	60	3.50	4,650	0	11	17	0	0	0	35	1	10	10	7	0
3/25/40	60	3.33	7,150	0	12	11	0	1	0	45	1	4	8	9	0
3/26/40	57	3.30	6,700	0	10	9	0	1	7	52	2	7	9	3	0
3/27/40	57	3.35	6,900	0	5	5	0	0	0	68	3	1	6	3	0
3/28/40	56	3.29	9,700	0	8	4	0	0	5	73	1	2	5	3	0
3/30/40	55	3.00	11,800	0	7	4	0	0	1	74	0	5	6	3	0
4/ 1/40	52	2.95	11,750	0	6	4	0	0	2	75	0	2	9	2	0
4/ 4/40	50	2.79	9,100	0	7	3	0	0	2	74	2	0	9	3	0
4/ 5/40	53	3.23	6,200	0	4	5	0	0	2	78	0	1	9	1	0
4/ 6/40	52	3.08	5,550	0	5	3	0	0	0	80	1	1	8	1	1
4/ 8/40	52	2.99	4,650	1	5	1	0	0	4	76	0	2	7	4	0
4/10/40	50	2.94	4,450	0	4	5	0	0	3	74	1	3	5	4	1
4/12/40	52	3.00	5,000	0	6	4	0	1	2	77	2	1	5	2	0
4/13/40	55	3.40	9,950	0	5	6	0	0	2	79	0	0	4	4	0
4/15/40	70	3.54	46,000	1	3	3	0	0	2	83	3	1	3	1	0

white woman, with impaired bilateral expansion, impaired resonance, and harsh breath sounds in both apexes. A roentgenogram of the chest showed exaggerated hilar markings with infiltration of both apexes, characteristic of active bilateral tuberculosis. The sputum was positive for tubercle bacilli. Other tests gave essentially negative results. The initial blood count was normal except for slight leukocytosis (12,650 white cells per cubic millimeter). No abnormal or immature blood cells were found. In April bilateral pneumothorax was instituted, and the patient's progress was uneventful until March 20, 1940, when she complained of pains in the lower part of the abdomen and backache, irregular menstrual bleeding and fever. A blood count at this time revealed marked anemia and leukopenia with 43 per cent immature monocytes. Repeated blood counts showed progressive anemia, leukocytes ranging from 3,500 to 12,000 per cubic millimeter and an increasing number of monocytes (table 1).

TABLE 2.—*Character of the Bone Marrow*

	A 1,000 Cell Differential Count on the Patient's Sternal Marrow, Aspirated, One Week After Onset of Leukemia (Per Cent)	Differential Count on Normal Sternal Marrow (Average Per Cent)
Myeloblasts	0.5	0.5
Promyelocytes	0.9	3.5
Neutrophilic myelocytes	1.2	1.0
Juveniles	1.7	8.0
Band forms	1.3	32.0
Segmented forms	0.8	17.0
Basophilic myelocytes	1.0	0.1
Basophils	2.0	0.2
Eosinophilic myelocytes	0.2	0.2
Eosinophils	0.5	1.5
Erythroblasts	0.2	1.5
Macroblasts	1.2	9.0
Normoblasts	4.5	6.5
Monocytoid cells	81.5	4.5
Megakaryocytes	0.1	0.2
Lymphocytes	2.4	14.0

At this time there was no enlargement of glands, but the spleen was palpable, and there was diffuse tenderness over the entire abdomen; the uterine cervix was soft and the uterus slightly enlarged and bleeding. The patient appeared toxic, had a rapid pulse, persistently high temperature (100-102 F.) and progressive weakness. She began to bleed from the gums and nose, and hemorrhagic areas developed in the skin. A sternal puncture at this time showed markedly hyperplastic bone marrow.

The bleeding time was eleven minutes, fifteen seconds; the coagulation rate, five minutes, thirty seconds; the platelet count, 47,200; the prothrombin time, 40 per cent of normal; the reticulocyte count, 4 per cent. An agglutination test for heterophil antibodies was negative.

The diagnosis was acute monocytic leukemia.

Six blood transfusions (1,600 cc.) were given over a period of fourteen days. Vitamins K and C were administered in an attempt to check the bleeding. Roentgen therapy was considered inadvisable.

The patient became progressively worse: The gums became more swollen and markedly hyperemic and partook of a purplish maroon color, with the appearance

of painful necrotic lesions, 2 to 3 mm. in diameter. She became extremely sensitive to light, and on April 14 it was noticed that the pupil of the right eye was markedly dilated, while that of the left eye was very small. Right hemiplegia developed. On April 15 she became comatose and died. A blood count taken one hour before death showed leukocytes 46,000 with 88 per cent immature monocytes.

THE BLOOD

The anemia was the type usually found in the leukemias. The color index ranged from 0.8 to 1.0. The majority of the erythrocytes were normal in size but slightly deficient in hemoglobin. However, some microcytes and polychromasic macrocytes, a few poikilocytes and basophilic stippled cells were found. A few nucleated red cells were present in all the smears, with an increasing number during the last ten days, averaging 2 per hundred leukocytes. A slight decrease in the number of thrombocytes was noticed from the beginning, with progressive thrombopenia (47,000 per cubic millimeter on April 1, 25,000 per cubic millimeter on April 15). There was a tendency toward subleukemia, the counts ranging from 3,900 to 12,000 per cubic millimeter, until one hour before death, when the count went up to 46,000.

The lymphocytes were all of the normal variety, shifting but slightly to the left.

There occurred a moderate shift to the left in the neutrophils, with an almost complete absence of eosinophils and only a few basophils. From 30 to 50 per cent of the neutrophils were band forms with an occasional older metamyelocyte. *No typical young myelocytes or promyelocytes* were found in any of the blood films.

THE MONOCYTES

For convenience and to provide some conception as to the range of immaturity of the monocytes, these cells were divided into four groups, and to facilitate a better understanding of their origin, they are presented in the reverse order of age.

Group 4 includes mature monocytes, varying from those with the typical horseshoe-shaped nucleus to those with an extensively lobulated nucleus, embedded in a "ground glass" bluish gray cytoplasm. Mature monocytes made up 6 to 8 per cent of the total number of leukocytes during the first few days, gradually decreasing to 1 per cent on the last count.

The cells of group 3 seemed to be in transition between those of group 2 and the mature monocytes. They all showed pseudopod formation, and the nucleus, although still somewhat sievelike, approached the more normal thready nucleus of the mature monocyte. The cytoplasm had the typical ground glass appearance but was somewhat more basophilic than normal.

The cells of group 2 were predominant, their number ranging from 40 to 80 per cent of the total number of leukocytes. These cells exhibited an immature sievelike nucleus with a few indistinct nucleoli. The nucleus was fairly transparent and had the appearance of being folded or wrinkled. Some of these cells showed one fold; others, as many as four or five. Still other forms contained double or triple nuclear lobes which

overlapped each other, some showing an indistinct nucleolus in each lobe. The cytoplasm was muddy in appearance and basophilic, and contained fine azurophilic granules which tended to gather at the edge of the cytoplasm and in the bay or cleft of the nucleus. In some cells the zone of cytoplasm was quite wide; in others it was fairly narrow, with the nucleus blending into the cytoplasm rather than standing out sharply defined. The majority were slightly peroxidase positive. The most outstanding characteristic was the pseudopodia or scalloped edges.

Group 1 includes the youngest forms found. These showed a moderately fine sievelike nucleus with an occasional indefinite nucleolus. The cytoplasm was moderately basophilic and usually did not disclose any granulation. Although morphologically indistinguishable from myeloblasts, they were considered to represent an older type of monoblast because of the association with various stages of immature monocytes

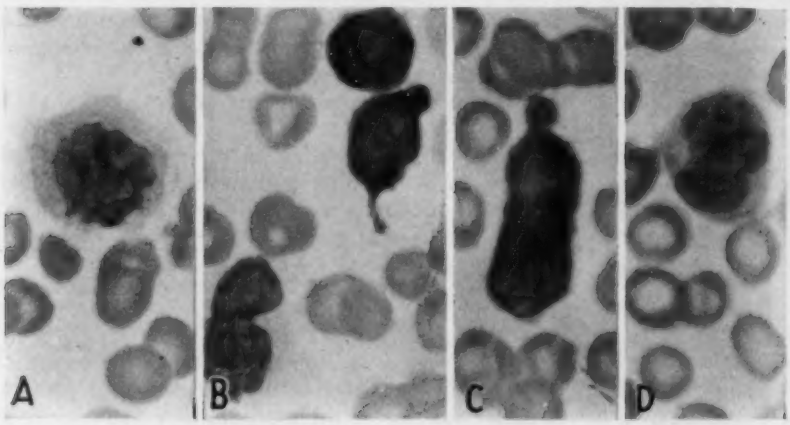

Fig. 1.—*A*, "monoblast." *B*, three "monocytoid" cells. Note the pseudopod formation. *C*, "monocytoid" with a pseudopod. *D*, "young" monocyte. All, × 1,170; Wright's stain.

and also because the granulocytes *did not shift any further back than the metamyelocytic stage.*

THE BONE MARROW

In normal marrow the ratio of myelopoiesis to erythropoiesis is approximately 3.7 : 1.0. Usually about 4.5 per cent monocytes may be found. In our case the myelopoietic-erythropoietic ratio was 1.7 : 1.0, with monocytoid cells as high as 81 per cent of the marrow elements at the beginning, increasing to 95 per cent at death. There was a preponderance of monocytoid cells that *diluted but did not change* essentially the ratios of the granulocytic or erythropoietic groups. Neither did it shift these cells far to the left. A significant feature, however, was that the "myeloblasts" were not diluted in the same way as the other cells, suggesting that they were largely monoblasts and that they promptly changed to monocytoid cells. Morphology may thus not offer a final criterion of function.

GROSS PATHOLOGIC FINDINGS

The body was that of a fairly well nourished white woman, 26.7 years of age, 120 pounds (54.5 Kg.) in weight and 5 feet 5 inches (165 cm.) in height. The upper gums were swollen, purple-red and hemorrhagic. There were many ecchymoses, measuring up to 1 cm., beneath the skin of both arms, in the scalp and over the chest. There was an old infraumbilic operative scar.

The midline fat in the abdominal wall was 1 cm. in thickness, the liver was 1 fingerbreadth below the costal border, and the serosa of the stomach, intestines and abdominal wall was studded with petechial hemorrhages. The great omentum was adherent to the old operative scar, and the vermiform appendix was absent.

The diaphragm on both sides reached the fifth rib, the lungs were slightly collapsed, the pleura was thickened over the domes of both lungs, and there were fibrous adhesions along the mediastinal borders.

There were petechial hemorrhages on the epicardium and pericardium and about 60 cc. of clear straw-colored fluid in the pericardial sac.

The heart weighed 270 Gm. The myocardium was red-brown and firm, with an occasional pea-sized irregular hemorrhage deep in the muscle. Otherwise the cardiovascular system was normal.

There were several 5 mm. calcifications in the lower portion of the upper lobe of the right lung and smaller ones in the corresponding hilar lymph nodes. There were several calcifications measuring up to 2 mm. scattered in the upper lobe of the left lung. In the base of the upper lobe of the right lung was a firm atelectatic and fibrotic area, 3 by 2 cm., with a whitish center and a pigmented periphery. Near it was a 3 mm. calcified nodule. A smaller but similar area was seen in the apex of the right lung. The upper lobe of the left lung was rather firm, subcrepitant and atelectatic. In its midportion was a carnified firm area, 2 by 1 cm., with a central 4 mm. caseous portion showing slight calcification. Smaller calcifications were also near. In the apex of the left lung was a sharply defined wedge-shaped purple area. Small purplish areas were seen also in the upper lobe of the right lung. In both lungs these areas represented recent hemorrhage in old lesions.

The liver weighed 2,025 Gm. and was smooth, grayish brown and firm, with the superficial and deep markings indistinct.

The spleen weighed 605 Gm. and was reddish purple, smooth and firm. The capsule was bound to the left lobe of the liver and diaphragm by firm fibrous adhesions. The section markings were indistinct, but many irregular yellowish gray infarcts measuring up to 3 cm. were visible.

Throughout the body the lymph nodes were only slightly enlarged.

The kidneys together weighed 365 Gm. and appeared normal except for a few 2 mm. whitish nodules.

The stomach revealed petechial hemorrhages along the greater curvature beneath the mucosa, and the intestines also had several submucosal hemorrhages measuring up to 8 mm. in the ileum and transverse colon.

The uterus contained a soft thin clot in the corpus. In the vagina were submucosal petechial hemorrhages with several round shallow gray ulcers measuring up to 3 mm.

The brain weighed 1,315 Gm. There were loose clots, homogeneous, soft, deep red, in the subdural space. The cerebral vessels were distended.

Marrow from the middle portion of the right femur was grayish red and firm.

The other organs were essentially normal.

MICROSCOPIC OBSERVATIONS

The splenic capsule was fairly normal. Beneath the capsule were numerous areas of hemorrhage, and deep in the pulp the large infarcts were composed of seminecrotic cells. The lymphoid tissue of the malpighian bodies consisted of only a few cells around the arteries. In certain areas there were cells undergoing active proliferation into monocytic cells. It was difficult to determine their origin. In the walls of overdistended sinusoids were an equal number of blood corpuscles and monocytic cells, which occupied only about 25 per cent of the intervening spaces. In the young cells there were various stages of cell division. The cytoplasm was usually pinkish and not overly abundant, and the nucleus was round, oval or, sometimes, indented. The inner structure could not be determined, because of the density of the stain.

The marrow was cellular and its structure obliterated. Only a few capillaries were visible, with a packing of monocytoid cells in between. The cells were oblong to reniform, with densely staining nuclei and sparse cytoplasm. Occasionally a giant cell was present, but only after much search could a myeloid cell be found. There was also interstitial hemorrhage, as in practically all other organs.

The structure of the lymph nodes was obliterated. There was free blood in the subcapsular sinuses, decreasing in amount toward the medulla. There was no sharp distinction between the germinal centers and lymphoid tissue, but many mitotic figures appeared in the reticular cells and young lymphocytes in the germinal centers. There were many large free cells in the sinuses, resembling lymphocytes. They were loosely mixed with red cells. In the medulla were many foci of monocytoid cells surrounding the blood vessels, similar in appearance to those in the other organs.

The outline and the arrangement of the liver cords appeared normal. The cytoplasm of the older liver cells, however, consisted of only a few scattered granules with here and there patches of a more normal-appearing cytoplasm. The more young and vigorous cells appeared in pairs. In these cells the nuclei were round, clear and normal in staining and revealed every stage of mitosis. So prominent were these mitotic figures that at first they gave the impression of a malignant process. The Kupffer cells were prominent, and some were undergoing active proliferation. The sinusoids contained a normal amount of red blood cells and a few monocytic cells. These usually had kidney-shaped or indented nuclei with a small amount of cytoplasm, and averaged about 15 microns in diameter. In the periportal area the monocytic cells were quite numerous and crowded the blood vessels and bile ducts into spaces smaller than normal. The Kupffer cells in the region of these foci of monocytes were quite prominent, but the origin of the monocytic cells was not certain.

In the kidney, mitotic figures were seen among the convoluted tubule cells, but not to the same extent as in the liver. A few small cystic adenomas of tubular cells were present. All other structures appeared normal.

In the lungs there was a ten to twelve year old primary lesion with many more recent calcifications. There was bilateral fibroid tuberculosis with fibroid tissue and alternating atelectasis and emphysema. In the healed and healing portions were many areas of free blood. There was also slight emphysema throughout both lungs. In the portion of the lung not involved in tuberculosis the alveoli were practically normal in size but marked stimulation of the cells of the alveolar walls was evident. These cells were more numerous than usual and were being extruded into the lumen, where many appeared as dust cells.

In the thyroid gland the alveolar spaces were filled with normal pink-staining colloid. Only in a few places was there any disappearance of colloid or hyperplasia of the cells. There was a suggestion of very early toxic changes.

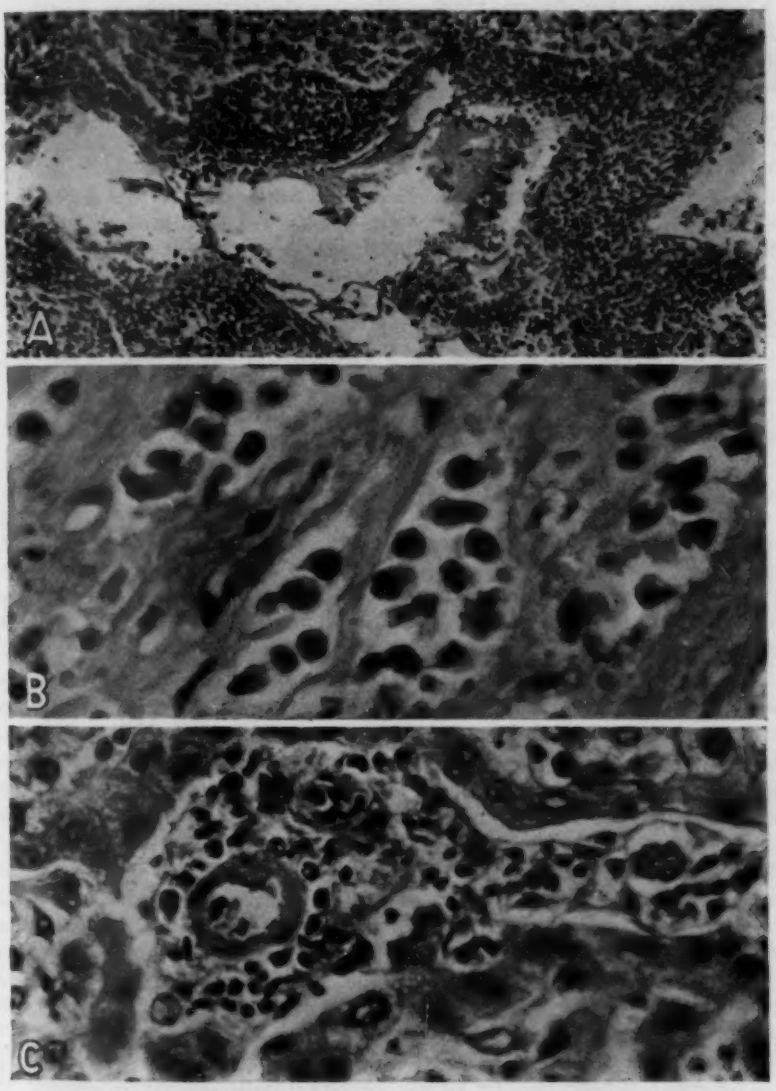

Fig. 2.—*A*, blood vessel in the medulla of a lymph node with perivascular infiltration; × 130; hematoxylin and eosin. *B*, section of a viable part of the spleen, showing monocytes in sinusoids; × 650; hematoxylin and eosin. *C*, periportal region of the liver, showing monocytic infiltration; × 430; hematoxylin and eosin.

The adrenal gland showed some destruction of the adrenal cells and little evidence of replacement. The cords in the cortex were separated by spaces in which

there was some fibrous tissue. The outermost layer of cells was considerably disintegrated, and there were many vacuoles in the cytoplasm. In the medulla the lipoid disintegration of cells was much more marked. Only about 30 per cent of the cell content remained.

In the gum tissue the important feature was the total necrosis of all the cells in the crater of the ulcer that extended from the surface to the periosteum. It raised the question whether the source of the ulcer was not in the changes in the marrow underneath.

The vagina had an irregular, extremely roughened epithelial covering. In some places it was completely denuded, and in other places there were sharp papillary projections. Beneath the mucosa were many accumulations of cells, most of which had the appearance of reticulum cells, but some were free monocytes of various sizes and shapes. Deep in the muscularis were mixed thrombi in some of the arteries and veins. In some of the lymphatic channels beneath the mucosa there were a few large multinuclear giant cells.

In the intestines there was moderate edema, with considerable disintegration of the superficial layers of the mucosa, but no deep ulcers.

The cardiac muscle was normal, but areas of free blood lay between the muscle bundles.

In the ovary clots of blood distended the more recent graffian follicles. One of these clots was as large as the organ itself.

Outside of occasional petechial hemorrhages of the brain, other organs and tissues did not reveal any significant changes.

COMMENT

This is the first case in which monocytic leukemia was reported as following tuberculosis. There is no evidence of a relation between the two conditions.

The case is also interesting from the standpoint of the apparent origin of the monocytes. Judging from the observations on the marrow, one may say that the "myeloblasts" were the only cells that were not diluted out by the disease cells and were the most logical source of the monocytes. Some day more refined chemical stains may enable one to divide such cells into myeloblasts and monoblasts, but thus far that is not possible. They appear like myeloblasts, and Downey [14] and others say they are myeloblasts. The liver, lymph nodes and pulmonary alveoli showed moderate proliferation of the "modified" endothelial cells, but there was no detectable connection with the monocytes. There was also proliferation of many other body cells, as in the liver and kidneys, which indicated a powerful stimulus of unknown origin. The reticulum was not unduly prominent anywhere, but in the spleen there appeared to be a slight amount of proliferation.

It may seem to be unwarranted to discuss here such a specialized and controversial subject as the origin of blood cells, but it is of distinct advantage in order to clarify the diagnosis and to give the most probable origin and lineage of the cells present. The old battle royal of blood cell genealogy reminds one of the fable of the chameleon. Every investigator is probably right in some respects but sees the problem in a slightly different light or studies his objectives under slightly different conditions. Some reports are on normal blood cultured and stained in

different ways; some on pathologic blood; others on normal animal tissues, and still others on tissue and blood cultures.

The most controversial point seems to be the question of *fixed* or *variable* cell ancestors. The monophylites believe in a single stem cell origin with an environment of special stimuli acting on the stem cells to direct them into the many known forms. To use football nomenclature, the stem cell is a sort of roving center that goes in as the play (stimuli) directs. The arguments for this theory are many, especially those established on studies of abnormal tissues (infected tissues, malignant growths, tissue cultures). Embryonic cells have widely different potentialities. Investigators surely have not yet been able to demonstrate all the functions of a given morphologic cell unit. Even more mature end cells may sometimes vary widely. Witness the changing of fibroblasts into chondroblasts and osteoblasts, and the reputed change of endothelium, reticulum, histiocytes and osteoblasts (deprived of vitamin C) into fibroblasts. Some of the cytologic transformations from "lymphocytes" to other blood cells reported by Maximow and Bloom [19] would be almost incredible under more normal conditions as studied by the supravital staining methods of Sabin and her school.

The polyphylites (including the dualists and the trialists), establishing their evidence more on "normal" conditions, seem not to obtain so much effect of abnormal environments and are prone to observe a more orderly development. They see, therefore, a more fixed position of the cells and the cell ancestors.

In favor of this view is the fact that there is always a set number of cell types that develop irrespective of the stimuli applied. Although one type may be exaggerated over the others, it is rare that any form may not be identified instantly. If there were not a rather fixed lineage of all cells, why should there not appear aberrations entirely out of the known pathways of development, as occurs in malignant growth?

Of all the blood cells, the monocytes seem to be the most controversial as regards origin. Forkner [20] listed nineteen different theories of origin having in view about six main cell types. The majority focus attention on some cells related to the basic reticulum. If there are two types of monocytic leukemia, as contended by Downey and others, there must be at least two sources of the monocytes, one the reticulum and one the "myeloblasts." But where do the "myeloblasts" originate? Most agree on a stem cell, but may that not also come from reticulum? There is still much work to be done to settle the controversy.

To reconcile the views it may be suggested that there are fixed cell species normally and that even under abnormal conditions these normal pathways may be followed. But it must also be admitted that environment may play a role in the development of cells to meet nature's requirements, that the greater the abnormality the greater may be the aberration and that not all the potentialities of the cells of the adult connective tissue, their origins and fate are known. Furthermore, investigators have not

19. Maximow, A., and Bloom, W.: Textbook of Histology, Philadelphia, W. B. Saunders Company, 1930, p. 127.

20. Forkner, C. E.: Arch. Int. Med. **34**:1, 1934.

even begun to explore the "jungles" of the embryonic tissues of the body. Until the many views are reconciled, or as they become reconciled, it will be necessary to maintain a middle position.

Apart from many theories and facts, however, there are definite end results, one of which is the clearcut case just reported.

SUMMARY

A case of monocytic leukemia having a clinical duration of twenty-five days in a patient convalescing from pulmonary tuberculosis has been described. The patient was followed with complete studies from two years before the onset to death, including a complete autopsy. The cell of origin resembled a myeloblast and was so designated until subsequent examinations revealed that monocytes of different degrees of development were the only abnormal cell element. The logical deduction, therefore, was that the cells of origin were *monoblasts*. The monocytic cell development appeared to take place chiefly in the marrow and spleen, with questionable minor changes in the lymph nodes, liver, lung and kidneys. An active growth of the parenchymal cells of the liver and kidneys also occurred as a result of an *unknown powerful stimulant*.

The most severe collateral changes, resulting perhaps from a disturbance of endothelium and thrombocytes, was hemorrhage of varying degrees in the spleen, marrow, lymph nodes and brain and in and beneath most of the mucous surfaces. Another resulting change was the development of small ulcers in the gums and in the vagina. The theories of cell origin have been discussed.

NEURILEMMOMA OF THE STOMACH WITH PEPTIC ULCER

RALPH H. FULLER, M.D., CINCINNATI

A 61 year old white woman was admitted to the hospital May 10, 1938; her chief complaints were of recurrent epigastric pain, nausea and vomiting of blood. She had first begun to suffer epigastric pain in December 1933. The attacks came at irregular intervals but often two or three times in the course of a week and usually about two hours after the last meal. The pain was frequently accompanied by nausea and vomiting. The symptoms were regularly relieved by self administration of alkali. Six months after the onset there was a sudden exacerbation of symptoms, the attacks of pain occurred more frequently, and on one occasion she vomited "a pint of dark blood." It was observed at that time that the feces became "tarry." She placed herself under the care of a physician, who prescribed a soft diet and rest in bed for three weeks. On recovering from that attack she abandoned the soft diet and shortly thereafter began again to suffer occasionally epigastric pain after meals. One year later, after another exacerbation of symptoms, she vomited blood and was bedfast for one week. During the following three years there was no hematemesis, but she continued, at irregular intervals, to suffer epigastric pain after meals. The attacks did not increase in severity, were regularly relieved by alkalinization of the stomach and often occurred several times over a period of several weeks, after which during a like period she would be almost symptom free. One week prior to her admission to the hospital there occurred for the third time an exacerbation of symptoms; nausea and epigastric pain began to occur regularly about two hours after each meal. On the day prior to admission it was noted that the feces were "tarry." That evening she experienced sharp epigastric pain, became nauseated and vomited "about a pint of material resembling coffee grounds." The next morning she suffered pain and nausea, vomited "about a pint of bright red blood," and felt "dizzy and weak." Aside from the history as narrated, no significant additional information was obtained.

She was a well developed and obese white woman about 60 years of age, mentally alert and in no pain. The temperature was 98.9 F.; the pulse rate, 84; the respiratory rate, 18; the systolic blood pressure, 124, and the diastolic pressure, 70. The skin was pale but dry and warm. There was no palpable enlargement of lymph nodes. The lung fields were "clear to percussion and auscultation." There were no physical signs of cardiac enlargement; however, the heart sounds were distant, and occasional extrasystolic contractions were noted. The abdomen was thick walled and pendulous. There was no abdominal rigidity. Tenderness to palpation was evident over the epigastrium and about the umbilicus. No abnormal masses were palpable. The pelvic examination revealed marked perineal relaxation and the presence of a large rectocele. The findings on neurologic examination were essentially normal.

The hemoglobin value was 14.4 Gm.; the erythrocyte count, 4,140,000; the leukocyte count, 12,650. The blood urea nitrogen content was 29 mg. per hundred cubic

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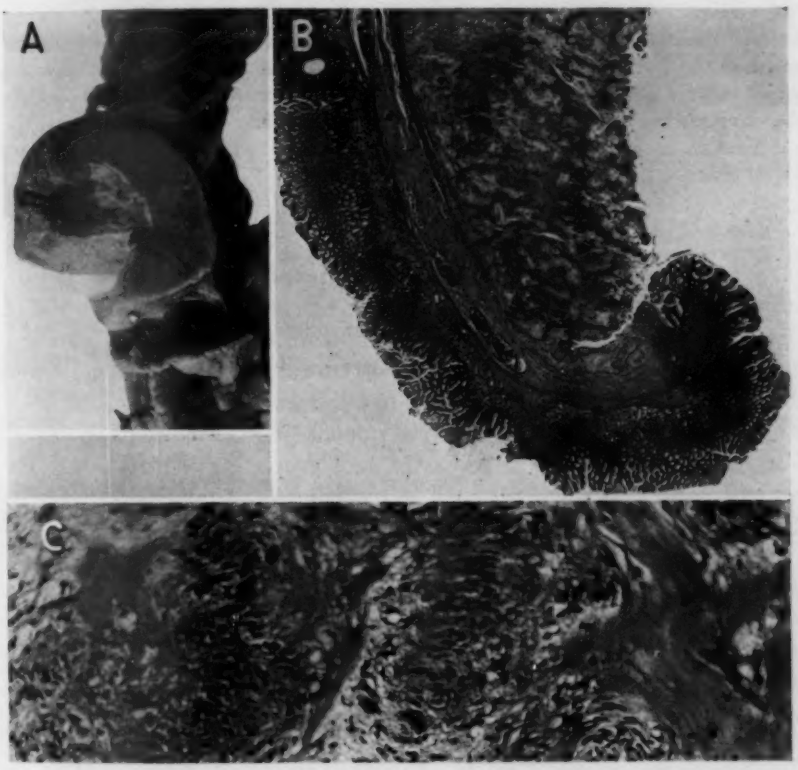
centimeters; the carbon dioxide-combining power 43 volumes per cent. Gastric analysis after fasting revealed a total amount of acid neutralized with 14 cc. of tenth-normal sodium hydroxide but absence of free acid. After administration of histamine phosphate the total acid readings at fifteen minute intervals were 62, 108, 95 and 76; the free acid readings, 36, 83, 82 and 55. Roentgen examination of the gastroenteric tract revealed the esophagus to be normal in caliber and contour. The stomach was somewhat hypertonic and normal in outline except for a rather large ulcer niche, about 2 cm. in diameter, lying on the posterior wall of the pars media. The rugae extending to this area were not deformed, and no palpable mass was made out through the abdominal wall in this region. There was some tenderness over the ulcer. Peristaltic activity could not be made out in this region, although at a lower level peristalsis was quite active. The duodenal cap was well visualized and appeared normal. The second and third portions of the duodenum were "not remarkable." At the end of twenty-four hours, no residue remained in the stomach, the large bowel was well filled with barium sulfate and appeared normal, and the appendix was freely movable and not tender. The lesion in the stomach showed none of the criteria of malignant neoplasia; the roentgenologic impression was one of gastric peptic ulcer. Gastroscopy was performed, and the report was as follows: "The pylorus was not seen. The antrum was not remarkable. At about the angulus on the lesser curvature a deep ulcerative lesion was seen, with somewhat raised and irregular edges. The base of the ulcer could not be seen. It appeared to be deep and penetrating. The lesion was sharply defined, and its upper margin was about 9 cm. from the cardiac end of the stomach. There was no evidence of inflammation in the remainder of the body of the stomach. The cardia was not remarkable. The impression is: malignant ulcer (Schindler type II). The lesion is operable; resection of the stomach is advised regardless of the roentgenologic findings."

The patient was placed on a Meulengracht dietary regimen. During the first week the erythrocyte count fell gradually to 2,320,000. As a preoperative procedure, a series of blood transfusions were given. Laparotomy was performed May 26, 1938. Exploration of the stomach revealed a "hard, cradle-like" ulcer at the juncture of the upper and middle thirds of the stomach on the posterior wall near the lesser curvature. Inspection of the regional lymph nodes revealed no evidence of metastatic neoplastic disease. "Sleeve resection" of the stomach was done. The postoperative course was uneventful, and the patient was discharged from the hospital in good condition, June 16, 1938.

Pathologic Examination.—The specimen received for examination in the laboratory was a strip of stomach wall measuring 15 cm. in length by 5 cm. in width. This appeared to be a resected cross section of the stomach laid open along the line of omental attachment at the greater curvature. Buried in the wall of the stomach, overlying the line of mesenteric attachment at the lesser curvature, was an encapsulated spheroid solid tissue mass measuring 3 cm. in diameter. The substance of the tumor was firm, gray and homogeneous. The cut surface did not present the characteristically whorled appearance of interlacing bundles of fibers cut in various planes so commonly seen in myoma and fibroma. There was no evidence of displacement of serosal or serosal-supporting tissue by the mass, which projected into the lumen of the stomach to produce a smoothly rounded hillock having a broad circular base. The gastric mucosa, only loosely attached to the capsule of the tumor mass, was everywhere of normal thinness and intact save at the apex of the hillock. Here

there was a "punched-out" oval ulcer, measuring 1 by 2 cm., without evidence of epithelial overgrowth at the ulcer margins. The ulcer crater perforated the capsule of the tumor and penetrated the substance of the tumor mass to a depth of 2 cm.

Microscopically, the tumor was composed of ribbon-like ranks of palisaded elongated nuclei. The ranks of nuclei appeared to branch and anastomose irregularly, presenting numerous whorls and eddies. By silver impregnation it was possible to demonstrate numerous silvered fibers, wiry in appearance, which were seen to pass across and through the ranks of palisaded nuclei to disappear in wide strands of hyaline substance which formed a coarse supporting tissue meshwork. This hyaline



A, portion of the surgical specimen showing neurilemmoma of the stomach with peptic ulcer. *B*, neurilemmoma of the stomach; hematoxylin–eosin; × 5.4. Note the perfect encapsulation of the tumor mass, the anastomosing ribbon-like ranks of palisaded elongated nuclei in the tumor substance and the erosion of the tumor related to ulceration of the overlying gastric mucosa. *C*, neurilemmoma of the stomach; hematoxylin–eosin; × 143. Note the characteristic histologic structure.

substance was in part collagenous and presented scattered small foci where calcium salts had been precipitated. The sparsely vascular tumor was perfectly encapsulated. There was a superficial marrow zone of necrosis and acute inflammatory reaction in the floor of the ulcer. No evidence of epithelial hyperplasia was to be seen at the margins of the ulcer. Sections of stomach wall in areas distant from the tumor

and ulcer presented no positive evidence of inflammatory change, although generally the superficial zone of lamina propria was richly cellular, plasma cells and eosinophilic leukocytes being especially numerous. The diagnosis was neurilemmoma of the stomach wall with active, essentially "peptic," ulceration of the overlying gastric mucosa and erosion of the tumor.

The patient returned periodically to the outpatient clinic for follow-up examination. Since she was found to remain in good condition, she was readmitted to the hospital, April 3, 1940, for perineal repair and uterine suspension. After this admission, roentgen reexamination of the stomach and upper part of the enteric tract was made. The esophagus was "negative." The rugae of the stomach were well seen. No abnormalities were noted about the stomach. The duodenal cap was well visualized. The duodenal loop was not enlarged. The small bowel pattern was normal. Perineorrhaphy was done April 24, 1940. From this operation the patient made a good recovery and was discharged from the hospital May 9, 1940. She was seen in the clinic at regular intervals during the next six months. At the last time she visited the clinic, Dec. 15, 1940, there had been no recurrence of gastric distress, and she remained in good condition.

COMMENT

The histogenesis of this nerve sheath tumor is a subject of controversy. Various terms have been applied to this neoplastic entity at various times by various observers and from varying points of view. It has been called perineurial fibroblastoma, neurinoma, schwannoma, lemmoma, neurilemmoma, and, when found in certain locations, cerebellopontile angle tumor or acoustic nerve tumor. The term "neurilemoma," proposed by Stout,[1] has in its favor both sound lexicography and the fact that it appears to be accepted by proponents of both major schools of thought as regards histogenesis.

Nerve sheath tumors are not always benign. However, when malignant neoplasia is shown, it becomes difficult certainly to differentiate the neoplastic process from mesodermal fibrosarcoma. Of course, the question as to whether malignant neurilemmoma and neurogenous (so-called neurogenic) sarcoma exist as separate entities must await a decision as to the histogenesis of neurilemmoma.

In the case of the tumor of the wall of the stomach reported here, the relatively high differentiation of tumor cells and tumor structure, the perfect encapsulation of the tumor mass, the relatively long clinical course of symptoms essentially those of peptic ulcer, and the recovery of the patient without evidences of recurrence support identification of the neoplastic disease as one of benign character.

Neurilemmoma is not a tumor of common incidence; certainly its occurrence in well differentiated typical form in the wall of the stomach is a rarity. The case reported, however, is of particular interest because it serves to illustrate the fact that neoplastic disease of the wall of the

1. Stout, A. P.: Am. J. Cancer **24**:751, 1935.

stomach may, through pressure on the overlying gastric mucosa or usurpation of the latter's blood supply, result in localized mucosal ulceration, a process which is essentially one of "peptic ulceration." The syndrome may be essentially one of peptic ulcer. Leiomyoma, a tumor which is probably of commoner incidence in the wall of the stomach, has quite similar potentialities. T. B. Mallory [2] has pointed out that in many cases of a small ulcerated carcinoma of the stomach, although the clinical history and the appearance of the specimen suggest that the carcinoma may have arisen from the margin of a preexisting peptic ulcer, the process is probably one of primary development of the neoplastic disease, which remains for a time localized in the mucosa but which lowers the local resistance so that the neoplastic mucosa becomes the site secondarily of "peptic ulceration." Benign neoplastic disease of the wall of the stomach may lead secondarily to "peptic ulceration" of the gastric mucosa, as was seen to occur in this case of neurilemmoma.

SUMMARY

A case is reported of neurilemmoma of the wall of the stomach complicated by ulceration of the overlying mucosa with erosion of the tumor. The clinical syndrome was one of peptic ulcer. The tumor and ulcer were resected, with subsequent recovery of the patient.

At least one article in the recent medical literature presents evidence in support of the theory that peptic ulcer showing an area of carcinoma at its margin does not represent carcinoma arising in a preexisting peptic ulcer but is actually a "carcinoma in situ" which becomes secondarily the site of "peptic ulceration." This case of neurilemmoma is presented as an instance of "peptic ulceration" secondary to benign neoplastic disease of the wall of the stomach.

2. Mallory, T. B.: Arch. Path. **30**:348, 1940.

Laboratory Methods and Technical Notes

A MODIFICATION OF MALLORY'S PHOSPHOTUNGSTIC ACID-HEMATOXYLIN STAIN FOR FORMALDEHYDE-FIXED TISSUES

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The excellent staining properties of Mallory's phosphotungstic acid–hematoxylin have long made it popular with pathologists working routinely with tissues fixed in Zenker's solution. The fact that this stain is also the simplest and yet one of the most consistently successful and brilliant technics for exhibiting neuroglia cells and their fibrils is less generally known. Doubtless this is due to the circumstance that all printed descriptions of the original method specify primary fixation of the fresh tissue in Zenker's fluid. On the other hand, neuropathologists employ alcohol or solution of formaldehyde U. S. P. for fixation almost exclusively, and tissues so fixed fail completely to stain with the technic originally prescribed by Mallory.[1a] A greater use of Zenker's fluid in the fixation of nervous tissue, especially tumors, is to be recommended, but formaldehyde solution is universally popular and indeed is the only practicable fixative for entire brains. Tissue from outside laboratories is almost invariably received in formaldehyde solution, so perforce the technic of staining rather than that of fixation must be altered to fit the circumstances.

From time to time a number of modifications have been suggested to obviate the necessity of fixation in Zenker's solution and to make the phosphotungstic acid–hematoxylin technic more generally available. Romeis [2] quoted Masson as recommending mordanting paraffin sections of material fixed in Bouin's or Helly's fluid in compound solution of iodine (Lugol's solution) for two to twenty-four hours, bleaching in 5 per cent sodium thiosulfate, washing in tap water and then staining overnight as usual in phosphotungstic acid–hematoxylin. While this report was in preparation, two additional methods applicable to mounted sections were published. Eichorn [3] mordants sections four hours in Zenker's fluid containing 5 per cent concentrated nitric acid. Mullen and McCarter [4] treat sections for two to twenty-four hours before staining in a solution containing 5 per cent chromium chloride and 5 per cent glacial acetic acid.

From the Division of Pathology, National Institute of Health.

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1. Mallory, F. B.: (*a*) Pathological Technique, Philadelphia, W. B. Saunders Company, 1938, p. 76; (*b*) p. 241.

2. Romeis, B.: Taschenbuch der mikroskopischen Technik, Munich, R. Oldenbourg, 1932, p. 199.

3. Eichorn, K. B.: Arch. Path. **31**:391, 1941.

4. Mullen, J. P., and McCarter, J. C.: Am. J. Path. **18**:289, 1941.

For staining of formaldehyde-fixed tissue, including tissue of the central nervous system, Kernohan [5] prescribes mordanting in Weigert's solutions before embedding. The desired blocks are cut from the specimen and washed in running water to remove the formaldehyde. After this they are mordanted for four days in Weigert's primary mordant and then two days in Weigert's secondary mordant. The blocks are then embedded in paraffin, and sections are stained according to Mallory's original directions.

Mallory [1b] more recently has suggested mordanting blocks of formaldehyde-fixed brain in a saturated aqueous solution of lead chloride for seven days at 37 C. The tissues are then washed in running water overnight and embedded in paraffin or celloidin (a pyroxylin preparation). Sections are treated with 5 per cent oxalic acid for thirty to sixty minutes, rinsed in tap water and stained overnight in phosphotungstic acid–hematoxylin as usual.

It is apparent that both the latter methods, though suitable for neuropathologic work, require treatment of fixed tissue before embedding. They are also time consuming, and may render tissue so treated less suitable for other staining methods. Since part of the specimens received in this laboratory have been submitted only in the form of paraffin-embedded blocks or sections, an attempt has been made to devise a method that would give consistent and satisfactory results on paraffin sections of formaldehyde-fixed tissue. Sections mordanted twenty-four hours in Zenker's solution or in 5 per cent iron-alum occasionally gave satisfactory preparations, but generally the stain was weak and inconstant. In the belief that the mercuric chloride of Zenker's fluid was the active mordanting agent, I tried to bring the sections in contact with the highest possible concentration of mercury. Saturated alcoholic solutions—both hot and at room temperature—proved unsuccessful. Their ineffectiveness in spite of the high concentration of mercury may have been due to the slight ionization in alcohol. Saturated aqueous solution at room temperature for twenty-four hours gave only a weak and irregular stain. When, however, sections were mordanted for several hours in a saturated aqueous solution of mercuric chloride in a paraffin oven at 57 C., the resulting stain was uniformly successful and compared favorably with that obtained after primary fixation in Zenker's solution.

After some trial, the optimum technic for formaldehyde-fixed tissues was determined to be as follows:

1. Deparaffinize sections and bring down to water.

2. Mordant three hours in a saturated aqueous solution of mercuric chloride in a paraffin oven at 57 C. Rinse briefly. The mercuric chloride solution may be used repeatedly.

3. Place in compound solution of iodine for five minutes. Rinse.

4. Place in 5 per cent aqueous solution of sodium thiosulfate for five minutes. Rinse.

5. Place in 0.25 per cent potassium permanganate for five minutes. Rinse.

6. Place in 5 per cent aqueous oxalic acid for five minutes. Wash well.

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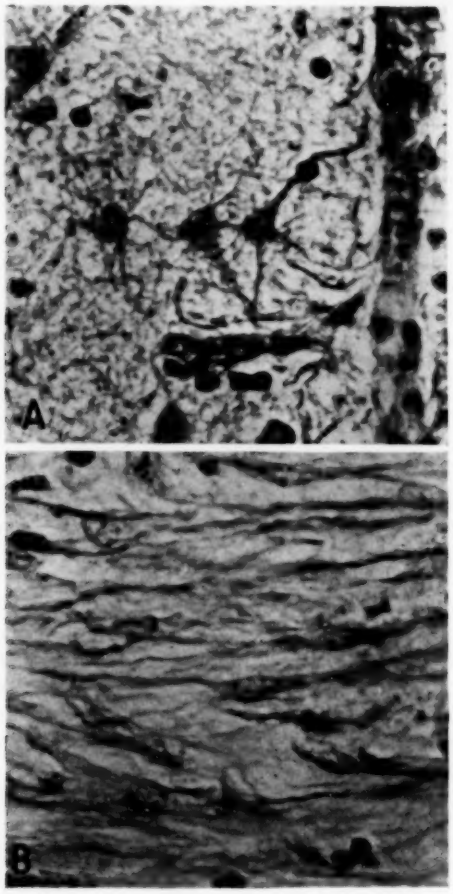
5. Kernohan, J. W.: Am. J. Clin. Path. **1**:397, 1931.

7. Stain over night in phosphotungstic acid–hematoxylin.

8. Wash briefly in tap water, dehydrate in alcohol or acetone, clear in xylol and mount in balsam.

This technic gives results almost identical with those of the original method as applied to tissue fixed in Zenker's solution. Neuroglia, fibroglia and myoglia fibrils, blepharoplasts, nuclei, red blood cells and



A, fibrous astrocytes near edge of tumor; × 515.
B, fibroblasts showing fibroglia fibrils from granulation tissue; × 515.

fibrin are deep blue, while collagen is brownish red. Two photomicrographs show neuroglia and fibroglia fibrils stained by this technic.

Mordanting for three hours seems to produce uniformly satisfactory results. If mordanting is carried on for less than two hours, the preparations are often poor, especially if the tissue was not originally fresh and well fixed. Increasing the time up to eight hours seems to make the stain more intense but less clean and precise. Longer mordanting

may be tried with advantage if that for the usual three hours proves insufficient. This preliminary treatment with mercuric chloride is also of marked value in improving the staining of occasional Zenker-fixed tissues, especially animal tissues, that stain feebly with the original technic.

Finally it must be emphasized that the foregoing method is not proposed as one to supplant the original Mallory technic, but only as a compromise alternative when Zenker-fixed tissue is not available. At best the results fall somewhat short of the crispness and clarity of preparations made from Zenker-fixed material. Its success is directly determined by the preservation of the tissue and the delicacy of the embedding. Satisfactory results cannot be expected from partly autolyzed or badly shrunken specimens.

SUMMARY

In order to make possible staining of formaldehyde-fixed tissues with Mallory's phosphotungstic acid–hematoxylin, it is suggested that paraffin sections be mordanted for three hours in hot saturated aqueous solution of mercuric chloride. This technic has been described in detail. Several earlier technical modifications for the same purpose have been noted and discussed.

SUGGESTED MODIFICATION OF THE MANDLEBAUM METHOD OF EXAMINING BODY FLUIDS FOR CELLS

SIEGFRIED TANNHAUSER, M.D., TUCSON, ARIZ.

A routine procedure in many pathologic laboratories now is the embedding in paraffin of the centrifugate of a body fluid such as pleural exudate or ascitic fluid and examination of this centrifugate for particles of a malignant growth (Mandlebaum [1]). Recent publications testify to the practical value of the procedure. Often, however, when the centrifugate is scanty, there is difficulty in keeping it together in a small enough space to allow all its parts to be studied. It also happens that after a 10 per cent solution of formaldehyde has been added to the centrifugate for the fixation of the particles and cells, precipitation occurs in small brittle fragments instead of in one large piece; these fragments later break up even more when they become harder in the graduated alcohols. Often one has to centrifuge continuously in order to keep a sizable amount of fragments together. Since the centrifuging has to be done at low speed in order not to destroy the cells, the centrifugate remains loosely packed and parts of it may get lost in the washings.

To collect the centrifugate as quantitatively as possible for the final embedding, the following modification has proved valuable. It also cuts down on the time and work connected with continuous centrifugation of the particles:

DESCRIPTION OF THE MODIFIED METHOD

The fluid (pleural or ascitic) is collected with addition of an appropriate amount of an anticoagulent (sodium citrate or potassium or sodium oxalate). After centrifuging the mixture in a large tube with a flat round bottom, a suitable amount of oxalated or citrated plasma is added; double the quantity of the remaining centrifugate has been found appropriate. If, for instance, an oxalated plasma, obtained by adding 0.4 cc. of 4 per cent potassium oxalate to each 10 cc. of blood, is used, 10 drops of a 0.5 per cent solution of calcium chloride for each cubic centimeter of plasma will induce clotting. It is important to add foreign plasma and not to rely on the clotting properties of the body fluid, since often this will not clot, owing to previous spontaneous defibrinization. The centrifugate is thoroughly mixed with the plasma, and calcium chloride is added. In a short time clotting starts and encloses all cells present in the centrifugate. After retraction, the clot is transferred to a 10 per cent solution of formaldehyde or Zenker's fluid for fixation, and its further treatment is that of any other specimen, with the fibrin of the clot holding the cells together in a satisfactory way.

When oxalated or citrated plasma is not available, a few centimeters of freshly obtained venous blood may also be used to advantage.

From the Pathological Department of the Desert Sanatorium.

1. Mandlebaum, F. S.: J. Lab. & Clin. Med. **2**:580, 1917.

The particles of malignant growth stand out well from the blood clot. In case an examination of the cellular content of the fluid is desired besides examination for cells or particles of malignant growth, that has to be done on a separate smear before addition of the blood.

This modification of the method of Mandlebaum may also be used when examination of sputum for malignant cells is to be made and paraffin embedding of the whole sputum is desired. The procedure then differs somewhat from that described:

In order to prevent disintegration of the cells by the action of the always present bacteria, the sputum is directly collected in a 10 per cent solution of formaldehyde. Usually the mucous sputum balls are dissolved or precipitated in small fragments by the formaldehyde (except when the sputum is highly albuminous; it then is more coherent after fixation with a solution of formaldehyde alone). After fixation of the sputum for twenty-four hours, the particles are centrifuged and washed twice with distilled water; a suitable amount of plasma with calcium chloride solution is then added. The resulting clot is again transferred to a solution of formaldehyde or Zenker's solution for fixation, washed and dehydrated in the graduated alcohols as already described.

Forensic Medicine

SIGNIFICANCE OF DEXTROSE AND NONDEXTROSE REDUCING SUBSTANCES IN POST-MORTEM BLOOD

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The pathologist and more frequently the medical examiner is confronted occasionally with cases of sudden death or of finding a person dead in which the clinical and laboratory data obtained prior to death are either inadequate or entirely lacking. In some instances the presence of one or more diseases makes the problem of determining the principal cause of death difficult, while in other cases no striking pathologic lesions may be observed. Among the many conditions occasionally responsible for sudden death with autopsy observations not always striking are diabetic coma, poisoning and asphyxia. Sugar determinations made on appropriately selected specimens of blood in such cases frequently provide useful evidence. Inasmuch as conditions present in the cadaver differ from those present during life, a knowledge of the various modifying factors as they pertain to blood dextrose is essential before the result of determinations of blood sugar are evaluated.

The purpose of this investigation was to attempt to answer three separate questions. These were:

1. From what part of the body should a postmortem sample of blood be taken for analysis?

2. What factors alter the dextrose content of the blood after death?

3. Of what significance are the results from determinations of dextrose and nondextrose reducing substances in blood removed after death?

SELECTION OF THE SAMPLE FOR ANALYSIS

It is obviously essential that the sample for analysis should be selected from a situation where an artefact is least likely to give rise to misleading results.

This potential source of error, so often overlooked, was found to be of the utmost importance in the present investigation inasmuch as the dextrose content of the blood rises in some parts of the body and falls

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in other parts after death (Hamilton-Paterson and Johnson [1]; Rathery and de Traverse [2]).

Interest in this phenomenon was aroused early in the investigation by the study of a case of acute cardiac failure in which blood obtained from the left ventricle by cardiac puncture two hours after death contained 110 mg. of dextrose per hundred cubic centimeters, while blood removed from the right auricle twenty-four hours later contained 418 mg.

The rate of the postmortem rise in blood sugar in the right auricle was observed in normal and in fasting dogs killed under controlled experimental conditions. The details of the experiments are as follows:

Experiment 1.—Normal and fasting dogs were anesthetized with pentobarbital sodium and sufficient heparin was administered intravenously to prevent clotting. By cardiac puncture, approximately 100 cc. of blood was removed, and the animal was then killed by air embolism. The chest was immediately opened, the heart exposed and all blood remaining in its chambers removed and mixed with the blood previously taken. This blood was reinjected into the chambers of the right and left sides of the heart until they were distended. Samples of blood for analysis were then removed periodically from the right auricle and the left ventricle. Sugar was determined by the Folin-Wu method, which in common with all methods utilizing the oxidation reduction principle also reports as sugar certain nonfermentable but reducing substances. These nondextrose reducing substances vary in normal blood from 10 to 30 mg. per hundred cubic centimeters and average about 17 mg. The fermentation method of Van Slyke [3] was used to determine the nondextrose reducing substances.

Representative experimental data are presented graphically in chart 1. In the case of the normal animal the sugar content of the auricular blood increased rapidly after death to a level over 400 mg. in two hours and to 720 mg. at the end of twenty-four hours. The sugar content of the blood from the left ventricle (not shown in the graph) fell from 93.5 to 28.7 mg. in five hours. The nondextrose reducing substances averaged 14.5 mg. in all samples and did not vary during the course of the experiment.

No postmortem rise in the sugar content of the blood of the right auricle occurred in the animal which had fasted for three days prior to the experiment.

Experiment 2.—The previous experiment was repeated on normal dogs in which all vessels to and from the heart were doubly ligated. Samples of blood for analysis were removed from the right auricle and the inferior vena cava at approximately hourly intervals.

Representative data are presented graphically in chart 2. Comparison of the blood sugar curve of the right auricle with that of the inferior

1. Hamilton-Paterson, J. L., and Johnson, E. W.: J. Path. & Bact. **50**:473, 1940.

2. Rathery, F., and de Traverse: Compt. rend. Soc. de biol. **128**:737, 1938.

3. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, Williams & Wilkins Company, 1932, vol. 2, p. 479.

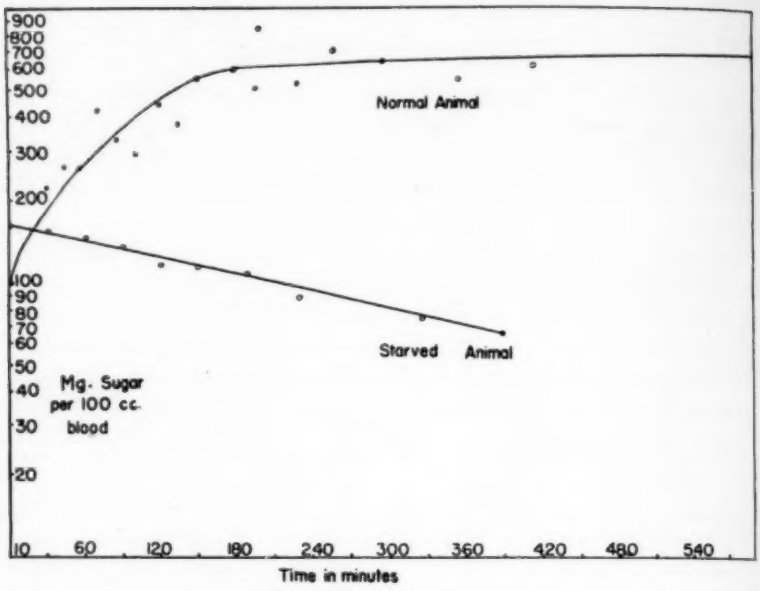

Chart 1.—Comparison between postmortem blood sugar levels in the right auricles of a normal and of a fasting dog.

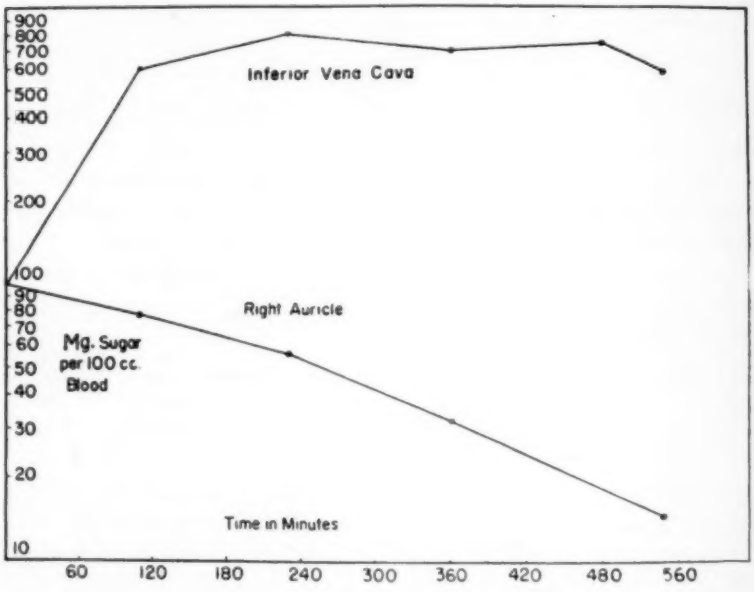

Chart 2.—Comparison between the blood sugar levels of the right auricle of an isolated heart and the inferior vena cava at varying periods after death.

vena cava indicates that the sugar responsible for the previously observed postmortem rise in the right auricle came from the inferior vena cava.

The notion that the postmortem rise in blood sugar in the right auricle might come from a release of cardiac muscle glycogen is not tenable. If this idea were correct, a greater rise in sugar would be expected in the left ventricle, where the myocardium is thicker and contains more glycogen per unit area.

Balachowsky and Ginsburg [4] observed that the cardiac blood of normal dogs several hours after death invariably showed a high sugar content, while a postmortem rise failed to develop in hepatectomized animals. They concluded that the liver was the source of the sugar causing this increase.

The rate of glycogenolysis in suspensions of minced liver tissue at 37.5 C. was investigated by Barrenscheen.[5] During the first two to four hours glycogenolysis occurred in a steplike manner and not in the form of a linear curve. The maximum rate of glycogenolysis occurred during the first hour, with the liberation of 108 to 343 mg. of dextrose, although it continued at a slower rate for many hours thereafter. An increase in the inorganic phosphate paralleled the release of dextrose and led to the conclusion that hexose monophosphate was produced.

Burghard and Paffrath [6] also reported that glycogenolysis occurred rapidly in isolated liver tissue and that there was no change in the total carbohydrate content of such tissue over a period of twenty-four to thirty-six hours. In such experiments the increase in dextrose paralleled the disappearance of glycogen (Simpson and Macleod [7]; Noltie [8]). That glycogenolysis can also occur in an atmosphere of nitrogen was demonstrated by Noltie.[8]

It seems probable that the increase in the sugar content of the blood of the inferior vena cava is due to the diffusion of dextrose from the liver through the hepatic veins to the inferior vena cava. The lungs apparently provide an effective barrier to the diffusion of sugar from the right side.

A comparison between the dextrose content of blood in the right and that in the left side of the human heart in 10 cases is shown in table 1. As would be expected from the experimental observations, there was a marked discrepancy between the dextrose content of blood removed from the right and that of blood removed from the left side

4. Balachowsky, S. O.; Ginsburg, F. S.; Farberowa, R.; Palitzina, T., and Rzihina, S.: Biochem. Ztschr. **252**:370, 1932.

5. Barrenscheen, H. K.; Pany, J., and Berger, R.: Biochem. Ztschr. **229**:196, 1930.

6. Burghard, E., and Paffrath, H.: Klin. Wchnschr. **6**:1479, 1927.

7. Simpson, W. W., and Macleod, J. J.: J. Physiol. **64**:255, 1928.

8. Noltie, H. R.: Quart. J. Exper. Physiol. **24**:261, 1935.

of the heart. The postmortem rise of dextrose which occurred in blood from the right side of the heart is undoubtedly the result of hepatic glycogenolysis with diffusion of the liberated dextrose through the inferior vena cava.

That a postmortem rise in dextrose in human subjects does not always occur is shown in table 2. In this table are included the data in 5 cases in which there was severe damage of the liver. The results

TABLE 1.—*Comparison of Dextrose Contents of Blood Samples from the Right and Left Sides of the Heart*

Case	Hours Post Mortem	Blood from Right Auricle, Mg. Dextrose per 100 Cc. Blood	Blood from Left Ventricle or Aorta, Mg. Dextrose per 100 Cc. Blood
1	2½	94.0	57.5
2	4¼	264.0	0
15	7¾	108.1	0
73	8¾	1,010.0	0
6	11	232.5	0
9	13	202.2	0
11	14	22.6	10
13	16	0	0
14	20	20.1	0
35	35	312.0	187.3

TABLE 2.—*Dextrose Levels in Cases in Which There Was Marked Damage of the Liver*

			Blood from Right Auricle			Blood from Left Ventricle		
Case	Lesion	Hours Post Mortem	Sugar *	Nondextrose Reducing Substances	Dextrose	Sugar *	Nondextrose Reducing Substances	Dextrose
66	Acute yellow atrophy.....	4	....	....	....	28.2	18.3	9.9
67	Acute yellow atrophy.....	6	56.0	30.0	26.0	37.0	27.8	9.2
68	Chronic passive congestion....	8	29.0	22.8	6.2	30.7	24.0	6.7
69	Chronic passive congestion....	10	31.2	34.2	0	27.2	28.2	0
70	Chronic passive congestion....	23½	34.8	32.4	2.4	38.2	25.0	13.2

* The sugar content is expressed as milligrams per hundred cubic centimeters of blood as determined by the Folin-Wu method. This method reports as sugar the nondextrose reducing substances present in the blood.

are comparable to those obtained for fasting dogs. It can be assumed that the antemortem depletion of liver glycogen in these subjects was so great that postmortem glycogenolysis did not contribute a significant amount of dextrose to the blood in the inferior vena cava. These observations are in accord with those of Hamilton-Paterson and Johnson [1] and Rathery and de Traverse.[2]

It can be concluded that samples of blood should be taken from the left side of the heart if the true postmortem dextrose content is to be determined.

This fact does not appear to have been appreciated by Hamilton,[9] who reported extreme variations in blood sugars in a series of cases in which samples of blood from the right auricle were used. Schmidt and Carey[10] in a series of 33 cases used blood obtained by cardiac puncture, and it is uncertain as to whether arterial, venous or mixed blood was used for their sugar determinations.

FACTORS AFFECTING POSTMORTEM GLYCOLYSIS

A knowledge of the rate of dextrose destruction is essential to the present study if the significance of appreciable quantities of dextrose remaining in the blood after death is to be determined. With this in mind, a brief summary of some of the more pertinent factors affecting glycolysis is given. In addition, supplementary experiments are given to elucidate some of the factors still in question.

Bernard[11] in 1873 first observed that the sugar content of blood progressively decreases after removal of the blood from the body. The decomposition of dextrose is now believed to be an enzymatic reaction that follows the general laws governing these reactions in being sensitive to changes in the enzyme concentrations, the p_H, the concentration of the substrate and the temperature and to the presence of poisons.

EFFECT OF CONCENTRATION OF ENZYME

Since the first observations by Lépine[12] it has been repeatedly confirmed that glycolysis fails to occur in blood serum. Laking of the blood by destroying its cellular elements will completely stop the glycolytic reaction. Kawashima[13] demonstrated that the degree of glycolysis is inversely proportional to the degree of hemolysis regardless of the method by which it is produced. Glycolysis stops when hemolysis is complete. The degradation of dextrose thus appears to be primarily an intracellular reaction, dependent on the vital processes of living cells.

According to Kawashima,[12] the degree of glycolysis and the number of red cells parallel one another when different samples of the same blood are used. Macleod[14] stated that centrifuged corpuscles from the dog completely exhaust their dextrose content in one-half hour at body temperature, while in whole blood a period of two and one-half

9. Hamilton, R. C.: Arch. Path. **26**:1135, 1938.
10. Schmidt, E. G., and Carey, T. N.: Arch. Int. Med. **47**:128, 1931.
11. Bernard, C.: Compt. rend. Acad. d. sc. **83**:369, 1876.
12. Lépine, R.: Le diabète sucré, Paris, F. Alcan, 1909.
13. Kawashima, Y.: J. Biochem. **2**:131, 1922.
14. Macleod, J. J.: Physiol. Rev. **1**:208, 1921.

hours is required for the dextrose content to fall to one-half its initial value (Macleod and Pearce [15]).

Blood from patients with erythremia shows much more rapid glycolysis than normal blood (Cook and Somogyi [16]; Falcon-Lesses [17]).

A glycolytic retardation proportional to the red cell decrease is present in pernicious anemia (Goldhamer [18]). Pathologic conditions involving active blood regeneration, with nucleated forms present, show a greater rate of glycolysis than normal blood (Burger [19]).

Löwy and Richter [20] in 1897 made the observation that glycolysis was lessened in leukopenia and increased in hyperleukocytosis. Pus was strongly glycolytic (Macleod [14]). Degradation of dextrose by the leukocyte was shown by Levene [21] and co-workers to be due to the new formation of lactic acid.

Although both red and white cells contain a glycolytic ferment, it has been demonstrated that the leukocytes play the dominant role. According to Maclean and Weir,[22] the ratio of the glycolytic activity of white to that of red cells varies between 200 and 1,000 to 1. Later work by Hsu [23] revealed that the ratio was 1,000 to 1 or, more specifically, that each leukocyte breaks down 8×10^{-9} mg. of dextrose per hour and each red cell only 7×10^{-12} mg. per hour.

As might be expected, leukemic bloods show an elevated rate of glycolysis. In myelogenous leukemia Falcon-Lesses [17] found that the rate of glycolysis was two to three times as rapid as normal so that the process was often complete in two to three hours. Similar results were obtained by Schmitz and Glover [24] and also Horsters.[25] In addition, these authors studied chronic lymphatic leukemia, observing that glycolysis approached normal except when immature forms were present.

EFFECT OF CONCENTRATION OF HYDROGEN IONS (p_H)

The glycolytic reaction can proceed over a wide range of p_H, extending from p_H 6 to 9, with an optimum p_H at 7.5 (Rona and Wilenko [26]). Other authors have reported the optimum p_H to vary

15. Macleod, J. J., and Pearce, R. G.: Am. J. Physiol. **32**:184, 1913-1914.
16. Cook, J. E., and Somogyi, M.: Arch. Int. Med. **44**:813, 1929.
17. Falcon-Lesses, M.: Arch. Int. Med. **39**:412, 1927.
18. Goldhamer, S. M.: J. Clin. Investigation **12**:583, 1933.
19. Burger, W.: Arch. f. exper. Path. u. Pharmakol. **150**:298, 1930.
20. Löwy, A., and Richter, P. F.: Klin. Wchnschr. **34**:1029, 1897.
21. Levene, P. A.: J. Biol. Chem. **11**:361, 1912; **12**:265, 1912; **14**:149 and 551, 1913.
22. Maclean, H., and Weir, H. B.: Biochem. J. **9**:412, 1915.
23. Hsu, F.: J. Physiol. **84**:173, 1935.
24. Schmitz, H. L., and Glover, E. C.: J. Biol. Chem. **74**:761, 1927.
25. Horsters, H.: Ztschr. f. d. ges. exper. Med. **97**:479, 1936.
26. Rona, P., and Wilenko, G. G.: Biochem. Ztschr. **62**:1 and 437, 1914.

between 8.0 and 8.5 (Abraham [27]; Roche and Roche [28]; Irving [29]). The reaction is stopped by a p_H below 5.5 and over 9.0

During the course of the reaction dextrose is converted to lactic acid, but inasmuch as the zone of optional p_H is broad, the effect of a change of p_H during the course of the reaction is not pronounced (Irving [29]; Roche and Roche [28]).

EFFECT OF CONCENTRATION OF SUBSTRATE

The majority of workers have agreed that the rate of disappearance of dextrose is independent of the initial concentration of dextrose in the blood (Macleod [15]; Jervell [30]; Holboell [31]; Irving [32]).

COMPARISON BETWEEN INTRACARDIAC AND IN VITRO GLYCOLYSIS

The rates of intracardiac and in vitro glycolysis were studied to determine whether any difference exists between them. If intracardiac and in vitro glycolysis were found to proceed at the same rate, further studies of the effects of temperature changes and of clotting of blood on the rate of glycolysis could be carried out in vitro.

Experiment 3.—Dogs were prepared by the method described in experiment 2, and all vessels to and from the heart were securely ligated. Blood removed prior to death was reinjected into the heart, and a specimen was also placed in a tightly stoppered test tube. This tube was inserted into the thoracic cavity where it would be subjected to the same temperature conditions as the heart. Samples of blood were removed for analysis at regular intervals.

In this experiment glycolysis was observed to occur at approximately the same rate in the test tube as in the right auricle or the left ventricle (chart 3). The belief that further studies on glycolysis could be carried out in vitro appeared justified.

EFFECT OF TEMPERATURE

Rate of Glycolysis at 37.5 C.—The glycolysis occurring in 6 specimens of blood incubated at 37.5 C. was carefully followed over a period of several hours. The results of this study are expressed in table 3.

The average rate of the destruction of dextrose was found to vary between 10.1 and 16.5 mg. per hour and to average 12.8 mg.

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27. Abraham, A.: Ztschr. f. klin. Med. **104**:609, 1926; Klin. Wchnschr. **6**:456, 1927.
28. Roche, J., and Roche, A.: Compt. rend. Soc. de biol. **96**:361, 1927.
29. Irving, J. T.: Biochem. J. **20**:1320, 1926.
30. Jervell, O.: Acta med. Scandinav. **74**:221, 1930.
31. Holboell, S.: Compt. rend. Soc. de biol. **93**:1681, 1925.
32. Irving, J. T.: Biochem. J. **20**:613, 1926.

On the assumption that the degradation of dextrose to lactic acid is a monomolecular reaction, the reaction constant k was calculated using the following formula:

$$k = \frac{1}{t} hr \cdot \frac{a}{a - x}$$

in which t is the time expressed in hours, a is the initial dextrose con-

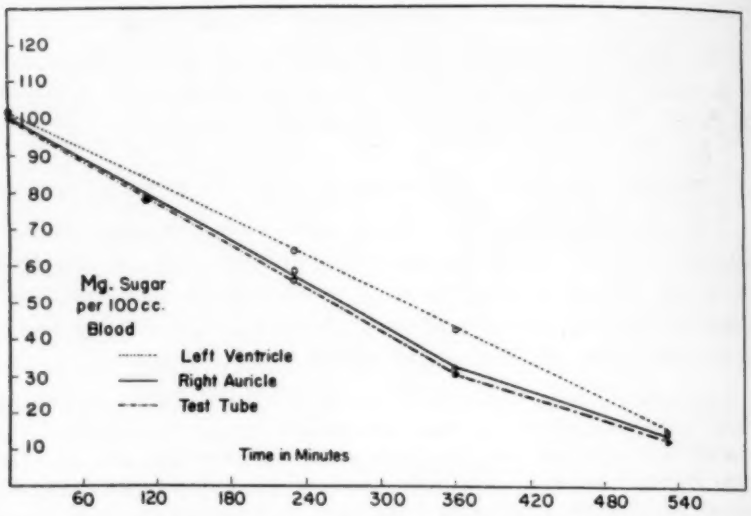

Chart 3.—Comparison between intracardiac and in vitro glycolysis.

TABLE 3.—*Glycolysis in Blood Samples at 37.5 C.*

Time, Minutes	No. 1		No. 2		No. 3		No. 4		No. 5		No. 6	
	Sugar *	Nondextrose Reducing Substances	Sugar *	Nondextrose Reducing Substances	Sugar *	Nondextrose Reducing Substances	Sugar *	Nondextrose Reducing Substances	Sugar *	Nondextrose Reducing Substances	Sugar *	Nondextrose Reducing Substances
0	104	14.0	107.0	11.8	115.0	15.7	100.5	17.6	93.0	...	100.0	15.0
60	98		88.6		101.5		94.5		78.3		81.4	
120	86		78.0		88.0		84.5		56.5		67.0	
180	70.4		68.5		74.0		65.0		42.0		42.7	
240	62.0		59.0		61.0		55.0		28.5		34.0	
300	52		49.0		49.0		44.0		18.0			
360	43		40.0		34.5		30.5		10.5	0.5		
420	33.3		30.5		21.0		18.0	16.7				
480	24.0		21.0		12.9	13.0						
540	15.9	15.0	14.4	12.4								
	10.1 mg. per hr.		11.1 mg. per hr.		13.6 mg. per hr.		11.7 mg. per hr.		13.71 mg. per hr.		16.5 mg. per hr.	
	K = 0.206		K = 0.205		K = 0.187		K = 0.182		K = 0.310		K = 0.280	

Average dextrose loss per hr., 12.8 mg.

Average K, 0.2283

* The sugar content is expressed as milligrams per hundred cubic centimeters of blood as determined by the Folin-Wu method. This method reports as sugar the nondextrose reducing substances present in the blood.

tent of the blood in milligrams per hundred cubic centimeters and $a - x$ the dextrose content of the blood after time t. To insure comparative results, k was calculated over the period of time required for glycolysis to proceed one-half way to completion.

The reaction constant was found to average 0.2283. Other papers dealing with the reaction constant are based on the incorrect assumption that the commonly used methods for the determination of sugar measure only dextrose.[33] The resulting error can assume fairly large proportions since the nondextrose reducing substances reported as sugar vary from 10 to 30 mg. per hundred cubic centimeters.

Effect of Changes in Temperature on Rate of Glycolysis.—Arthus [34] in 1891 reported the first studies on the effect of changes in temperature on the rate of glycolysis and noted that increases markedly accelerated the reaction. Lépine [12] in 1909 reported the optimum temperature of the reaction to lie between 38 and 39 C. and that heating the blood to 58 C. completely stopped the reaction. Birchard [35] in 1923 and Holboell [31] in 1925 confirmed the fact that lowering the temperature slows the reaction.

There have been few careful studies of the effect of changes in temperature on the rate of glycolysis. Hsu [23] reported a 23 per cent increase in the degradation of sugar when the temperature was increased from 33.5 to 38.5 C. Irving [32] in 1926 reported the temperature coefficient Q_{10} (between 27 and 37 C.) to equal 2.1 for rabbits' blood.

The rate of glycolysis in 5 specimens of citrated blood from normal human subjects which had been incubated at 27.5 C. was determined. The results are presented in table 4.

The rate of dextrose loss varied from 4.14 to 6.94 mg. per hour and averaged 5.98 mg. per hour.

The reaction constant, k, varied from 0.0978 to 0.1349 and averaged 0.1017. The temperature coefficient Q_{10} (between 27.5 and 37.5 C.) was found to equal 2.25 for normal human blood.

Under refrigerator conditions (3 C.) the average rate of glycolysis in normal blood was observed to be 1.08 mg. per hour.

EFFECT OF CLOTTING OF BLOOD

The comparative rate of glycolysis in citrated and in clotted blood was studied. Into each of sixteen clean test tubes 2 cc. of citrated blood was measured, and to eight of these tubes 1 drop of 5 per cent

33. Cajori, F. A., and Crouter, C. Y.: J. Biol. Chem. **60**:765, 1924.
34. Arthus, M.: Arch. de physiol. norm. et path. **3**:425, 1891.
35. Birchard, D.: J. Lab. & Clin. Med. **8**:346, 1923.

calcium chloride solution was added to permit clotting. Blood filtrates were prepared from each of the two series of tubes at hourly intervals.

The analytic results of this experiment are given in table 5. Glycolysis was found to proceed at approximately the same rate in clotted and in nonclotted bloods.

TABLE 4.—*Glycolysis in Blood Samples at 27.5 C.*

Time, Minutes	No. 1		No. 2		No. 3		No. 4		No. 5	
	Sugar *	Nondextrose Reducing Substances	Sugar *	Nondextrose Reducing Substances	Sugar *	Nondextrose Reducing Substances	Sugar *	Nondextrose Reducing Substances	Sugar *	Nondextrose Reducing Substances
0	100.0	12.4	104.0	14.8	107.0	11.5	87.0	11.8	111.0	15.2
60	94.0		100.0		101.5		81.0		104.5	
120	87.0		96.0		95.0		74.8		97.5	
180	80.5		92.0		89.0		63.9		90.2	
240	78.0		87.0		83.0		63.0		83.0	
300	67.0		83.0		76.8		57.0		76.4	
360	60.5		79.0		70.5		51.2		69.1	
420	54.0		75.0		64.3		45.0		62.0	
480	47.5		71.0		58.0		39.0		55.2	15.7
540	41.0	12.6	66.5		52.0		33.3			
600	36.0		63.0		46.0		28.0			
720	28.0		54.0	14.9	33.5	11.2	18.0	11.4		
	6.6 mg. per hr.		4.14 mg. per hr.		6.13 mg. per hr.		6.0 mg. per hr.		6.97 mg. per hr.	
	K = 0.0978		K = 0.0623		K = 0.1024		K = 0.1349		K = 0.1110	

Average dextrose loss per hr., 5.98 mg.

Average K, 0.1017

* The sugar content is expressed as milligrams per hundred cubic centimeters of blood as determined by the Folin-Wu method. This method reports as sugar the nondextrose reducing substances present in the blood.

TABLE 5.—*Glycolysis in Clotted and in Nonclotted Blood*

Time, Min.	Clotted Blood, Mg. per 100 Cc.	Nonclotted Blood, Mg. per 100 Cc.
0	93.0	93.0
60	75.0	75.6
120	61.5	60.0
180	49.0	49.9
240	37.5	40.0
300	29.5	31.0
360	20.0	22.8
420	11.0	14.0

SIGNIFICANCE OF DEXTROSE AND NONDEXTROSE REDUCING SUBSTANCES IN POSTMORTEM BLOOD

The dextrose and nondextrose reducing substances were determined in 73 medicolegal and hospital cases, and an attempt was made to correlate the findings with the causes of death.

A review of the literature indicated that glycosuria and hyperglycemia were often observed during life in cases of asphyxia, of shock

and of acute coronary occlusion and in cases presenting a rapidly developing anoxemia or increased intracranial pressure.

All such cases were classified separately regardless of the amount of dextrose present in the left side of the heart. For obvious reasons, cases of diabetes were classified together even though death was not necessarily due to this disease. For discussion purposes, cases of death from acute fluoride and cyanide poisoning and cases in which there were significant lesions of the liver were considered separately.

Cases not falling into the foregoing classification were used as controls. Among them were cases of cancer, of infection and of degenerative diseases.

Blood from the right auricle and left ventricle or aorta in these cases was analyzed for dextrose. The classification used in the following discussion is as follows:

1. Control cases.
2. Cases in which death was presumably due to asphyxia; i. e., cases of hanging, of strangulation and of the presence of foreign bodies in the pharynx and larynx.
3. Cases in which death was presumably due to anoxemia.
   (*a*) Cases of extensive hemorrhagic bronchopneumonia, of confluent bronchopneumonia, of massive lobar pneumonia, of pulmonary embolism and of massive collapse of the lung.
   (*b*) Cases of carbon monoxide poisoning.
4. Cases of diabetes regardless of the cause of death.
5. Cases in which increased intracranial pressure was apparent, i. e., cases of rapidly fatal cerebral hemorrhage and traumatic subdural hemorrhage.
6. Cases in which death was presumably due to shock following multiple injuries, such as fractures, lacerations and contusions of internal organs.
7. Cases of acute coronary occlusion.
8. Cases in which there were marked chronic passive congestion of the liver and toxic hepatitis.
9. Cases of death by poison: fluoride and cyanide.

Control Cases.—The 14 control cases reported in table 6 were used for the purposes of comparison. In 50 per cent of these cases death occurred under such circumstances as to require a medicolegal examination. Death did not occur suddenly or unexpectedly in the majority of these cases. The level of dextrose in the blood of the right auricle was moderately elevated in only 3 instances. This was not unexpected, as many of these persons were debilitated by their disease and their glycogen reserves were presumably low.

Popper and Wozasek [36] reported that liver glycogen was low in persons dying after any protracted illness in which there had been prolonged malnutrition or wasting of the tissues. The total glycogen content of moist liver in these cases ranged from 0.24 to 1.53 per cent, contrasting with values ranging from 1.56 to 6.17 per cent in cases of sudden death of a person previously in good health.

The amount of free dextrose present in blood of the left ventricle can be calculated by subtracting the milligrams of nondextrose reducing substances present from the milligrams of sugar. Free dextrose was insignificant in all cases in which the postmortem interval was over four

TABLE 6.—*Control Cases*

			Blood from Right Auricle			Blood from Left Ventricle		
Case	Cause of Death	Hours Post Mortem	Sugar *	Nondextrose Reducing Substances	Dextrose	Sugar *	Nondextrose Reducing Substances	Dextrose
1	Septic abortion	2½	118.0	24.0	94.0	83.5	26.0	57.5
2	Reticulum cell sarcoma	4¼	292.0	27.7	264.3	26.3	27.7	0
3	Cerebral hemorrhage, old	4½	29.0	30.7	0	32.0	30.1	0.9
4	Generalized tuberculosis	7¾	130.0	21.9	108.1	20.7	18.5	2.2
5	Subdural hemorrhage, old	10	36.2	29.4	6.8	24.1	21.3	2.8
6	Arteriosclerotic heart disease	10½	26.5	30.0	0	37.2	31.2	6.0
7	Carcinoma of rectum	11	265.0	32.5	232.5	32.5	28.7	3.8
8	Cerebral thrombosis	11	27.7	28.7	0	27.7	23.6	4.1
9	Multiple burns	11½	44.7	24.7	20.0	33.7	32.5	1.2
10	Veronal poisoning	13	226.0	23.8	202.2	23.8	24.1	0
11	Acute alcoholism	14	47.0	14.4	22.6	31.2	21.2	10.0
12	Meningitis	14½	29.6	24.5	5.1	22.6	23.2	0
13	Meningitis	16	27.5	24.0	3.5	27.2	23.8	3.4
14	Pericarditis	20	34.4	14.3	10.1	19.5	18.6	0.9
15	Incarcerated inguinal hernia	27	24.0	24.0	0	30.0	26.7	3.3

* The sugar content is expressed as milligrams per hundred cubic centimeters of blood and includes certain nondextrose reducing substances reported as sugar.

hours except 1. Hamilton-Paterson and Johnson [1] reported that complete glycolysis occurred in three and a half to seven hours after death in 8 cases in which the antemortem blood sugar was presumably within normal limits. Complete glycolysis was also observed by them in 24 similar cases in which the first examination was made seven to sixteen hours after death. The conclusion that the agonal blood sugar in the control cases lies within normal limits appears justified.

Cases in Which Death was Due to Asphyxia Following Obstruction of the Upper Respiratory Passages.—Eight cases of presumably asphyxial death are reported in table 7, and in 50 per cent of these the blood from the right side of the heart showed a markedly elevated dextrose

36. Popper, H., and Wozasek, O.: Virchows Arch. f. path. Anat. **279**:819, 1931.

level. Lesions supposedly characteristic of asphyxia were not necessarily present in all of these cases.

Hamilton-Paterson and Johnson [1] observed that six to seven hours elapsed before the temperature of the internal organs was appreciably lowered in bodies refrigerated shortly after death. During the period at which the internal organs were at approximately 37 C. glycolysis destroyed 12.8 mg. of dextrose per hour.

Appreciable quantities of dextrose, ranging from 28 to 608 mg., were present in 6 of the 8 cases reported in table 7. These observations were made six to nineteen hours after death. If allowance is made for the glycolysis which undoubtedly occurred before the internal

TABLE 7.—*Cases in Which Death Was Presumably Caused by Asphyxia Following Obstruction of the Upper Respiratory Passages*

			Blood from Right Auricle			Blood from Left Ventricle		
Case	Cause of Death	Hours Post Mortem	Sugar *	Nondextrose Reducing Substances	Dextrose	Sugar *	Nondextrose Reducing Substances	Dextrose
16	Foreign body in larynx	2½	418.0	14.0	404.0	111.0	13.0	98.0
17	Hanging	6	102.0	38.6	63.4	80.6	29.6	51.0
18	Strangulation	8	.....	....	....	56.0	28.0	28.0
19	Edema of glottis	8¼	26.0	24.4	1.6	15.8	15.8	0
20	Hanging	10	679.0	29.2	649.8	63.5	19.1	44.4
21	Hanging	16	678.0	29.0	649.0	634.0	25.4	608.6
22	Foreign body in pharynx	19	82.5	18.5	64.0	38.2	19.3	18.9
23	Hanging	20	169.0	27.2	141.8	26.4	24.7	0

* The sugar content is expressed as milligrams per hundred cubic centimeters of blood and includes certain nondextrose reducing substances reported as sugar.

organs had cooled, it becomes apparent that the agonal blood sugar in these cases must have exceeded normal limits.

No residual dextrose was found in 2 cases. One of these cases was that of suicide by hanging. It is possible that death may have been due to cerebral anoxemia rather than to asphyxia. In the other case marked edema of the glottis secondary to severe streptococcic pharyngitis was present at autopsy. Death in this case may have been due to the infection.

That asphyxia leads to hyperglycemia was first observed by Claude Bernard [37] in 1857 and has since been confirmed many times.

In view of the marked hyperglycemia present in case 21, an experiment was performed to determine the range of the hyperglycemic response to asphyxia.

37. Bernard, C.: Leçons sur les effets des substances toxiques et médicamenteuses, Paris, J. B. Baillière & fils, 1857.

Experiment 4.—An anesthetized dog was gradually asphyxiated by intratracheal administration of 3 cc. doses of liquid petrolatum. Samples of blood for analysis were taken by cardiac puncture after each injection of oil.

The resulting blood sugar curve is given in chart 4. It will be noted that with increasing asphyxiation the blood sugar rises rapidly to a level of 520 mg. just before death. The nondextrose reducing substances remained at 16.4 mg. during the course of the experiment.

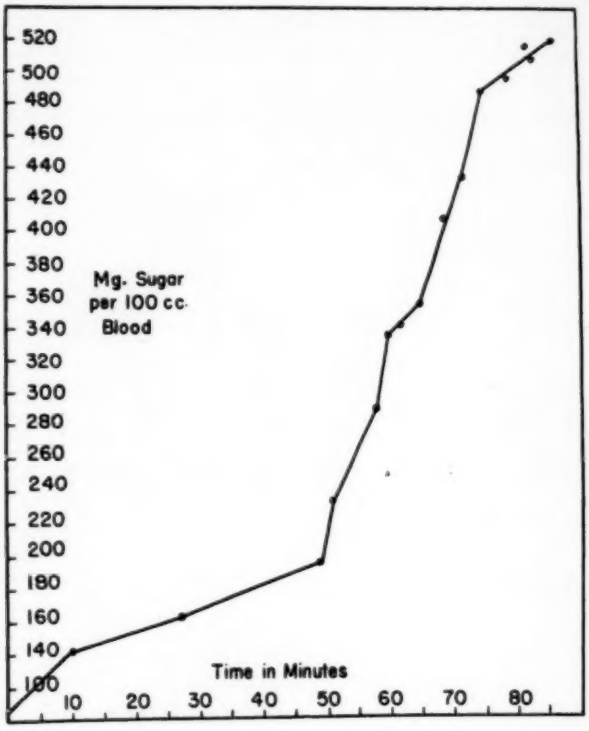

Chart 4.—Blood sugar curve during experimental asphyxia. Each observation was made after intratracheal administration of 3 cc. of liquid petrolatum.

Blood sugar levels of 400 to 500 mg. appear to be possible in cases of prolonged asphyxiation.

Cases of Anoxic Death.—(*a*) Carbon Monoxide Poisoning: In 4 of the 5 cases of acute carbon monoxide poisoning reported in table 8, the carbon monoxide was encountered in the course of fires; the fifth was a case of suicide by inhalation of illuminating gas. A sufficient degree of saturation of the blood with carbon monoxide to cause death was present in every case.

In 4 of the 5 cases appreciable quantities of dextrose remained in the blood from the left ventricle eleven to sixteen hours post mortem. That glycolysis occurs at its normal rate in blood saturated with carbon monoxide was first demonstrated by Kawashima [13]; similar experiments carried out in vitro by me confirm this observation. Undoubtedly, the agonal blood sugar in these 4 cases had been definitey elevated.

Hyperglycemia, with a blood dextrose even up to 336 mg. per hundred cubic centimeters, is apparently common in carbon monoxide poisoning, Umber [38] reporting it in 68 per cent of 1,000 cases.

Mikami [39] reported the degree of hyperglycemia and glycosuria in poisoned rabbits to be roughly proportional to the degree of anoxemia. Similar observations were made on men by Münzer and Palma.[40] Hyperglycemia occurring as a result of anoxemia is apparently due to the mobilization of glycogen from the liver, as Araki [41] demonstrated

TABLE 8.—*Cases of Carbon Monoxide Poisoning*

		Blood from Right Auricle			Blood from Left Ventricle		
Case	Hours Post Mortem	Sugar *	Nondextrose Reducing Substances	Dextrose	Sugar *	Nondextrose Reducing Substances	Dextrose
24	11	762.0	96.0†	666.0	244.0	52.0†	192.0
25	11	78.0	14.2	63.8	56.0	12.3	43.7
26	11	162.0	30.5	131.5	28.7	24.7	4.0
27	16	.....	....	.....	196.0	20.5	175.5
28	18	118.0	31.5	86.5	49.0	26.7	22.3

* The sugar content is expressed as milligrams per hundred cubic centimeters of blood and includes certain nondextrose reducing substances reported as sugar.

† The high figure for nondextrose reducing substances is presumably due to incomplete fermentation of the dextrose in this sample.

that starved animals which had lost their hepatic glycogen did not show glycosuria when exposed to carbon monoxide. Furthermore, Schulze [42] demonstrated successive decreases in the glycogen content of the livers of poisoned mice following each exposure. He expressed the view that this effect was ascribable to the action of the asphyxia on the adrenals.

(*b*) Lesions Interfering with the Aeration of the Blood: Twelve cases were studied, in which the postmortem interval ranged from five hours to thirteen days (table 9). Such extensive intrapulmonic lesions were present that the resulting anoxemia was undoubtedly a contributory cause of death. However, lesions characteristic of anoxemia were not necessarily present in all of these cases. The interference with oxygen exchange developed quite rapidly in 5 of the cases (31, 34, 35, 36, 38).

38. Umber, F.: Med. Welt **9**:889, 1935.
39. Mikami, S.: Tohoku J. Exper. Med. **8**:237, 1926-1927.
40. Münzer, E., and Palma, P.: Ztschr. f. Heilk. **15**:184, 1894.
41. Araki, T.: Ztschr. f. physiol. Chem. **15**:335, 1891.
42. Schulze, E.: Arch. f. exper. Path. u. Pharmakol. **180**:649, 1936.

The dextrose content of blood from the left ventricle in the latter cases ranged from 10 to 188 mg., contrasting with the somewhat lower dextrose content in the remaining cases. Without a doubt, the agonal blood dextrose in all of these cases except no. 37 was elevated above normal limits. The degree of hyperglycemia developed apparently depends on the rapidity with which anoxemia develops. Schmidt and Carey [10] reported a number of cases of bronchopneumonia and pulmonary edema in which the blood sugar contents at the time of death ranged from 130 to 219 mg. per hundred cubic centimeters.

TABLE 9.—*Cases in Which Death Was Presumbaly Caused by Anoxemia Following Intrapulmonic Obstruction of Respiratory Passages*

			Blood from Right Auricle			Blood from Left Ventricle		
Case	Lesion	Hours Post Mortem	Sugar *	Nondextrose Reducing Substances	Dextrose	Sugar *	Nondextrose Reducing Substances	Dextrose
29	Acute pulmonary edema	4¾	100.2	26.4	73.8	55.2	28.2	27.0
30	Lobar pneumonia	5	132.0	26.5	105.5	65.0	27.1	37.9
31	Hemorrhagic bronchopneumonia	10	157.0	26.3	130.7	138.0	26.3	111.7
32	Tuberculous pneumonia	10	80.0	14.2	65.8	42.5	14.2	28.3
33	Lobar pneumonia	11	31.7	20.1	11.6	31.7	19.5	12.2
34	Confluent bronchopneumonia	11½	....	....	....	122.0	28.0	94.0
35	Tuberculous pneumonia	12	35.2	27.4	7.8	47.7	23.4	24.3
36	Pulmonary embolus	12	38.0	27.7	10.3	41.7	30.7	11.0
37	Lobar pneumonia	12	41.6	37.8	3.8	40.8	37.2	3.6
38	Traumatic pneumothorax	15½	80.0	25.2	54.8	25.7	25.0	0.7
39	Extensive pulmonary tuberculosis	18	160.0	28.3	131.7	47.0	24.2	22.8
40	Hemorrhagic bronchopneumonia	35	350.0	38.0	212.0	228.0	39.7	188.3
41	Acute pulmonary edema	312	674.0	58.4	615.6	46.7	26.6	20.1

* The sugar content is expressed as milligrams per hundred cubic centimeters of blood and includes certain nondextrose reducing substances reported as sugar.

Cases of Diabetes.—Six cases of diabetes are reported in table 10 regardless of the cause of death. In 3 of these cases death occurred in the course of diabetic coma. The dextrose content of blood from the right and from the left side of the heart (except in case 46) was markedly increased to approximately the same level. A probable explanation of this observation is that depletion of hepatic glycogen is commonly present in fatal cases of diabetic coma (Warren [43]).

Recent studies have indicated that there is no difference between the glycolytic powers of normal and diabetic bloods in vitro (Chelle and Mauriac [44]; Tolstoi [45]; Cajori and Crouter [33]; Macleod and Pearce [15];

43. Warren, S.: The Pathology of Diabetes Mellitus, Philadelphia, Lea & Febiger, 1938, p. 117.

44. Chelle, L., and Mauriac, P.: Compt. rend. Soc. de biol. **76**:852, 1914.

45. Tolstoi, E.: J. Biol. Chem. **60**:69, 1924.

Hamilton-Paterson and Johnson [1]). Hamilton-Paterson and Johnson [1] reported that postmortem glycolysis occurred at a slower rate in the cadaver than in vitro. Even if this were not the case, there can be no possible doubt that the agonal blood sugar levels in these cases were definitely elevated above normal limits.

Cases of Increased Intracranial Pressure.—Four cases of massive cerebral hemorrhage and a case of traumatic subdural hemorrhage which

TABLE 10.—*Cases of Diabetes*

Case	Hours Post Mortem	Cause of Death	Blood from Right Auricle			Blood from Left Ventricle		
			Sugar *	Nondextrose Reducing Substances	Dextrose	Sugar *	Nondextrose Reducing Substances	Dextrose
42	14½	Coma	461.0	28.2	432.8	422.0	30.1	391.9
43	14½	Veronal poisoning	384.0	28.5	355.5	410.0	29.0	381.0
44	16¾	Coma	1,392.0	28.7	1,363.3	1,144.0	17.6	1,126.4
45	17	Pulmonary tuberculosis	482.0	43.0	439.0	416.0	50.0	366.0
46	21½	Arteriosclerotic heart disease	188.0	29.0	159.0	102.0	26.5	75.5
47	36	Coma	366.0	36.0	330.0	324.0	28.0	296.0

* The sugar content is expressed as milligrams per hundred cubic centimeters of blood and includes certain nondextrose reducing substances reported as sugar.

TABLE 11.—*Cases with Increased Intracranial Pressure*

Case	Hours Post Mortem	Blood from Right Auricle			Blood from Left Ventricle		
		Sugar *	Nondextrose Reducing Substances	Dextrose	Sugar *	Nondextrose Reducing Substances	Dextrose
48	4	208.0	90.2 ?	117.8	17.2	21.0	0
49	5	592.0	21.0	571.0	592.0	22.0	570.0
50	7	46.2	30.0	16.2	49.0	30.8	18.2
51	12	198.0	31.2	166.8	86.4	21.4	75.0
52	16	235.0	20.1	214.9	187.0	20.1	166.9

* The sugar content is expressed as milligrams per hundred cubic centimeters of blood and includes certain nondextrose reducing substances reported as sugar.

terminated fatally within a few hours are reported in table 11. A pronounced postmortem increase of dextrose was present in blood from the right auricle in 4 cases.

Significant amounts of dextrose remained in the blood from the left ventricle in 4 cases, while in 1 case (48) glycolysis had gone on to completion. That the agonal blood sugar levels must have been definitely elevated in these 4 cases is quite apparent. Glycosuria and hyperglycemia are relatively common findings in cases of injury of the head and in

cases of increased intracranial pressure from other causes (Hamilton-Paterson and Johnson[1]; Mock and de Takáts[46]). It has been suggested that the glycosuria in these cases is the result of a disturbance in the vegetative centers of the brain (Hausner and Hoff[47]).

Cases in Which Death Was Presumably Due to Shock.—Six automobile victims dying within a few hours after the accident are listed in table 12. Multiple fractures, contusions and lacerations of the viscera predominated in this group, although there was 1 case in which only a rupture of the urinary bladder had occurred. A marked postmortem rise in the blood dextrose on the right side of the heart was present in 3 cases.

Relatively large amounts of dextrose were present in the blood of the left side of the heart in all but 1 case (54), leading to the conclusion that the agonal dextrose must have been correspondingly increased.

TABLE 12.—*Cases in Which Death Was Presumably Due to Shock*

		Blood from Right Auricle			Blood from Left Ventricle		
Case	Hours Post Mortem	Sugar *	Nondextrose Reducing Substances	Dextrose	Sugar *	Nondextrose Reducing Substances	Dextrose
53	5	126.0	18.0	108.0	78.0	17.7	60.3
54	8	54.0	18.8	35.2	30.2	22.2	8.0
55	14	428.0	28.0	400.0	120.0	24.0	96.0
56	14	655.0	22.4	632.6	679.0	22.6	656.4
57	15	77.5	29.0	48.5	74.7	26.0	48.7
58	15½	366.0	28.7	337.3	150.8	26.0	132.0

* The sugar content is expressed as milligrams per hundred cubic centimeters of blood and includes certain nondextrose reducing substances reported as sugar.

Konjetzny and Weiland[48] reported glycosuria in 40.9 per cent of a series of 83 cases of fracture, which was of diabetic origin, in only 3.6 per cent. Funsten[49] reported hyperglycemia (dextrose content, 364 mg.) developing in a nondiabetic patient several hours after the patient had suffered multiple fractures and other injuries.

Cases of Coronary Thrombosis.—Seven cases of sudden death from coronary thrombosis are listed in table 13. Elevated levels of dextrose in blood from the right side of the heart were noted in 6.

Appreciable quantities of dextrose persisted in the blood of the left side of the heart in a similar number of cases. There can be no reasonable doubt that the agonal blood sugar levels must have been markedly elevated in these cases.

46. Mock, H. E., and de Takáts, G.: Ann. Surg. **90**:190, 1929.

47. Hausner, E., and Hoff, H.: Ztschr. f. klin. Med. **125**:493, 1933.

48. Konjetzny, G., and Weiland, W.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **28**:860, 1915; cited by Funsten.[49]

49. Funsten, R.: J. Bone & Joint Surg. **17**:769, 1935.

Eppinger [50] stated that glycosuria and hyperglycemia are not infrequent at the beginning of acute coronary closure. Transitory hyperglycemia and glycosuria were observed by Scherf [51] in 6 of 9 cases of coronary thrombosis. In 1 case the blood sugar on admission was 230 mg. but returned to normal after three days. Increases in blood sugar were produced in dogs by experimental closure of the coronary arteries (Hausner and Hoff [47]).

Cases in Which the Liver Was Damaged.—Five cases are reported in table 2 in which the livers presented either marked chronic passive

TABLE 13.—*Cases in Which Death Was Due to Sudden Coronary Occlusion*

		Blood from Right Auricle			Blood from Left Ventricle		
Case	Hours Post Mortem	Sugar *	Nondextrose Reducing Substances	Dextrose	Sugar *	Nondextrose Reducing Substances	Dextrose
59	4½	330.0	28.2	201.8	166.0	32.5	133.5
60	7	177.0	31.0	146.0	88.6	20.5	68.1
61	8	640.0	35.4	604.6	21.5	15.4	6.1
62	11	149.0	21.0	128.0	63.1	18.1	45.0
63	14½	161.0	24.0	137.0	45.0	30.0	15.0
64	20½	395.0	35.5	359.5	96.8	34.0	62.8
65	31	37.6	25.0	12.6	59.0	24.2	34.8

* The sugar content is expressed as milligrams per hundred cubic centimeters of blood and includes certain nondextrose reducing substances reported as sugar.

TABLE 14.—*Cases of Poisoning*

Case	Hours Post Mortem	Cause of Death	Blood from Right Auricle, Mg. Dextrose per 100 Cc.	Blood from Left Ventricle, Mg. Dextrose per 100 Cc.	Rate of Glycolysis at 37.5 C., Mg. per Hr.
71	6	Fluoride poisoning	317.5	183.0	5.0
72	10	Fluoride poisoning	87.0	82.6	3.2
73	8¾	Cyanide poisoning	977.4	0	

congestion or acute yellow atrophy. In none of these cases was an appreciable quantity of dextrose found in blood from the right auricle.

The purpose in presenting this small series of cases is to emphasize the fact that when pronounced damage of the liver is present, the blood sugar of the right side of the heart is not high post mortem. It is suggested that this is due to a decrease in the storage of hepatic glycogen. Popper and Wozasek [36] reported low hepatic glycogen in cases of cirrhosis of the liver in which death had occurred gradually from the disease and also in cases in which the liver was full of metastatic malignant growth.

Cases of Poisoning.—Two cases of fluoride and a case of cyanide poisoning are presented in table 14. The dextrose level in blood from

50. Eppinger, H.: Wien. klin. Wchnschr. **47**:210, 1934.
51. Scherf, D.: Wien. klin. Wchnschr. **46**:69, 1933.

the right auricle was elevated in the case of cyanide poisoning and in 1 case of fluoride poisoning. From these observations it can be assumed that neither the fluoride nor the cyanide ion has an inhibiting effect on postmortem hepatic glycogenolysis.

Blood was removed from the left ventricle at autopsy in both of the cases of fluoride poisoning and incubated at 37.5 C. for six and eighteen hours, respectively. The rate of glycolysis was found to be 3.2 and 5 mg. of dextrose per hour, respectively, contrasting with an average rate of 12.8 mg. observed in 6 cases in which poisoning was not a factor, indicating that the fluoride ion had inhibited the glycolytic enzyme. Ewig [52] observed that aerobic and anaerobic glycolysis were greatly inhibited in isolated tissue by hundredth-molar solutions of sodium fluoride. This effect is due to the fluoride ion and is reversible.

In view of the depressed rate of dextrose degradation and the elevated dextrose level of the blood from the left ventricle it becomes obvious that the agonal blood dextrose level must have been markedly elevated. Gautrelet and Mallié [53] and also Foit [54] noted transitory hyperglycemia and glycosuria in rabbits which had received subcutaneous or intravenous injections of sodium fluoride. Goldenberg [55] made similar observations in a kid which had received 0.06 Gm. of sodium fluoride per kilogram orally. However, according to Suekawa,[56] hyperglycemia does not occur in splanchnectomized rabbits after they have received injections of sodium fluoride.

In the case of cyanide poisoning, over 12 Gm. of commercial sodium cyanide had been taken by mouth with suicidal intent. At autopsy, eight and three-quarters hours post mortem, glycolysis had gone on to completion in the left ventricle. Kawashima [13] observed that cyanides have no influence on glycolysis in vitro as long as the concentration is not great enough to produce hemolysis. This case confirms Kawashima's observations. Hyperglycemia in cyanide poisoning has been reported by Lépine.[12] It is probable that in the present case death occurred too rapidly for hyperglycemia to develop.

SUMMARY

Postmortem samples of blood for determinations of dextrose should be removed from the left side of the heart if significant errors due to the postmortem diffusion of dextrose from the liver to the right side of the heart are to be avoided. The dextrose responsible for the postmortem

52. Ewig, W.: Klin. Wchnschr. **8**:839, 1929.
53. Gautrelet, J., and Mallié, H.: Compt. rend. Soc. de biol. **60**:714, 1906.
54. Foit, R.: Bratisl. lekár. listy **11**:17, 1931.
55. Goldenberg, L.: J. de physiol. et de path. gén. **26**:426, 1928.
56. Suekawa, T.: Mitt. a. d. med. Akad. zu Kioto **3**:142, 1929.

rise in dextrose on the right side of the heart is liberated by glycogenolysis occurring in the liver. Fasting animals or cadavers presenting marked hepatic damage do not show this postmortem rise in dextrose.

Glycolysis occurring after death causes a progressive lowering of the dextrose content of the heart's blood. Intravascular and in vitro glycolysis occur at approximately the same rate and are not influenced by clotting of the blood.

The rate of glycolysis was studied in vitro at various temperatures, and the temperature coefficient of the reaction was determined.

The dextrose and nondextrose reducing substances were determined in 73 medicolegal and hospital cases. Significant quantities of residual dextrose were present in the majority of cases of asphyxia, shock, acute coronary closure, rapidly developing anoxemia, increasing intracranial pressure and fluoride poisoning, indicating that agonal hyperglycemia was present.

For significant results in cases in which hypoglycemia is suspected, specimens of blood for analysis should be taken within two hours after death.

General Reviews

THE ADRENAL CORTEX

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ADRENAL DEFICIENCY

Removal of the cortex of the adrenal gland is followed by disturbances in the distribution and excretion of inorganic ions. The immediate cause of the symptoms of adrenal deficiency appears to be related to these disturbances, which ultimately involve hemoconcentration and inability of the vascular system to retain water even when water is given by intravenous injection.

The organ which manifests the greatest loss of function and furnishes one of the easiest avenues for study of adrenal deficiency is the kidney. The loss of renal function following adrenalectomy has a far reaching influence on the physiologic state which rapidly develops, but it is quite certain that the primary changes are not confined exclusively to the kidney.

There are modifications in the intestinal tract. The rate of absorption through the intestinal walls is progressively reduced, peristalsis is increased, and eventually diarrhea and loss of blood by the bowel indicate terminal stages of adrenal deficiency.

The fundamental importance of the changes in the distribution of inorganic ions is indicated by the fact that adrenalectomized animals are restored to a condition which in many respects approaches normal by the regulation of the intake of sodium, potassium and chloride ions. However, when food is not ingested, there are changes in carbohydrate metabolism which are serious and may be the cause of death. Certain of the hormones of the adrenal cortex have a specific effect on glyconeogenesis, the deposition of glycogen in the liver and the concentration of dextrose in the blood.

The changes in the distribution and excretion of inorganic ions and in carbohydrate metabolism which occur in adrenal deficiency are associated with alterations in the metabolic processes which involve fats and proteins. There is a progressive decrease in the metabolic rate up to the point of death. It is therefore not surprising to find a subnormal state in many physiologic processes, such as maintenance of blood pressure and of body temperature and resistance to stress, to long-continued muscular activity and to toxic substances.

From the Division of Biochemistry, Mayo Foundation.

PREPARATION OF EXTRACTS; CHEMICAL NATURE OF THE HORMONES OF THE ADRENAL CORTEX

For about thirty years after the separation of epinephrine from the medulla of the adrenal gland, all attempts to prepare an extract of the adrenal cortex which could be used in substitution therapy failed. During the decade between 1920 and 1930 it was shown that life could be maintained in adrenalectomized animals for many days without the administration of extracts of the adrenal cortex. These results threw doubt on the early experiments in regard to the potency of extracts,[1] but in 1929 and 1930 Swingle and Pfiffner[2] and Hartman and Brownell[3] prepared extracts of the adrenal cortex which beyond all question possessed physiologic activity. Not only did these extracts maintain life in adrenalectomized rats, cats and dogs but they also were able to bring back adrenalectomized animals from the severe state of collapse which followed within a few days the cessation of the administration of the extract. Since 1930 extracts of varying potency have been available for experimental purposes and clinical use.

The general principles which govern methods of extraction of the active substances were early recognized. The active material is neutral and soluble in both water and organic solvents. It is destroyed by alkali and is at least partially destroyed by heat. All methods for the extraction of the active material from the adrenal cortex include a primary step which provides for the precipitation of the proteins and much of the fat and lipids by extraction either with acetone or alcohol. The extract is concentrated and freed from material insoluble in water, and the active material is then separated from the water with benzene, ethylene dichloride or chloroform. The organic solvent is evaporated under vacuum and the active material redissolved in water.[4]

The crude extract can be further purified by fractionation into those compounds more soluble in benzene than in water. In the benzene are found corticosterone and dehydrocorticosterone, compound H, desoxycorticosterone, progesterone and other compounds. In the aqueous solution there are present steroid derivatives which contain five atoms of oxygen (compounds C, D, E, F, G and others). These can all be removed through crystallization from chloroform. All the compounds

1. Banting, F. G., and Gairns, S.: Am. J. Physiol. **77**:100, 1926. Marine, D., and Baumann, E. J.: ibid. **81**:86, 1927. Rogoff, J. M., and Stewart, G. N.: ibid. **84**:660, 1928. Stewart, G. N., and Rogoff, J. M.: ibid. **91**:254, 1929.

2. Swingle, W. W., and Pfiffner, J. J.: Science **72**:75, 1930.

3. Hartman, F. A., and Brownell, K. A.: Science **72**:76, 1930.

4. (*a*) Pfiffner, J. J.; Vars, H. M., and Taylor, A. R.: J. Biol. Chem. **106**: 625, 1934. (*b*) Cartland, G. F., and Kuizenga, M. H.: ibid. **116**:57, 1936. (*c*) Kendall, E. C., in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, p. 299

can be extracted from water with chloroform, but when the chloroform is concentrated to a very small volume and allowed to stand for some hours, the crystalline material separates. The fraction which is soluble in chloroform is further purified by solution in water, extraction with benzene, reextraction of the benzene with water and finally a second crystallization from chloroform. The distribution between water, benzene and chloroform may be repeated many times until a fraction is obtained which is soluble both in chloroform and in water but which cannot be crystallized under any conditions which have been tried. This material will be designated as the amorphous fraction.[5]

PHYSIOLOGIC EFFECTS OF THE EXTRACT OF THE ADRENAL CORTEX

The whole extract prepared as described will maintain the lives of adrenalectomized animals. They are normal when examined in regard to distribution of inorganic ions, carbohydrate metabolism and resistance to stress, such as cold or muscular activity, and they have as great resistance to toxic substances, such as histamine, as has the normal animal. Even after withdrawal of food the adrenalectomized rat treated with the whole extract can deposit glycogen in the liver and have normal concentrations of dextrose in the blood.

PHYSIOLOGIC EFFECT AND CHEMICAL STRUCTURE OF ACTIVE CRYSTALLINE COMPOUNDS FROM THE ADRENAL CORTEX

When the chemical and physiologic investigation of the adrenal cortex was begun, there was nothing to indicate the presence of more than one hormone of the adrenal cortex. As the work progressed, it became more and more difficult to ascribe the influence on inorganic constituents and water and on the metabolism of carbohydrate to a single hormone. It is the purpose of this paper to review the available experimental work and to show that the adrenal cortex produces a surprisingly large number of steroid derivatives, many of which are physiologically active. Some of these have a specific effect on the metabolism of carbohydrates.

The simplest structure which has been demonstrated to possess physiologic activity characteristic of the hormones of the adrenal cortex is progesterone. Progesterone is a steroid derivative, with an unsaturated ketone group $3,\Delta^{4,5}$, in which a two carbon side chain is

5. (*a*) Mason, H. L.; Hoehn, W. M.; McKenzie, B. F., and Kendall, E. C.: J. Biol. Chem. **120**:719, 1937. (*b*) Mason, H. L.; Hoehn, W. M., and Kendall, E. C.: ibid. **124**:459, 1938. (*c*) Wintersteiner, O., and Pfiffner, J. J.: ibid. **111**: 599, 1935; (*d*) **116**:291, 1936. (*e*) Reichstein, T.: Helvet. chim. acta **19**:29, 1935. (*f*) Steiger, M., and Reichstein, T.: ibid. **21**:546, 1938; (*g*) **20**:1164, 1937. (*h*) Reichstein, T., and von Euw, J.: ibid. **21**:1197, 1938. (*i*) Beal, D., and Reichstein, T.: Nature, London **142**:479, 1938.

attached to position 17 in the steroid nucleus. On position 20 there is a ketone group and at position 21 a methyl group. Progesterone will maintain the life of adrenalectomized rats, male and castrated female cats and ferrets.[6]

In desoxycorticosterone, in addition to the ketone group on the side chain there is a primary alcohol group attached to carbon 21 (fig. 1). This alphaketol grouping is present in all the substances of the adrenal cortex which have been shown to possess physiologic activity. Whether

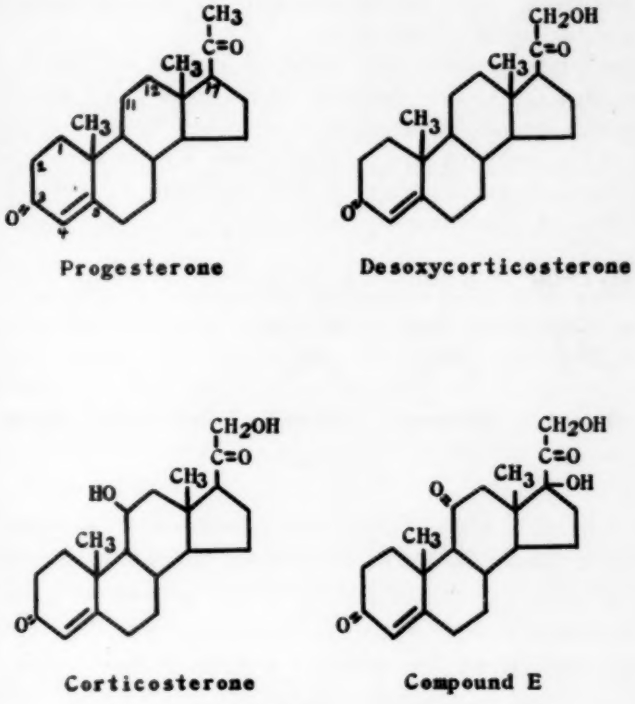

Figure 1.

progesterone is oxidized in the body so that the alphaketol grouping is produced is a question which has not been answered, but the reverse reaction in which the primary alcohol group is reduced to the methyl group has been shown by the conversion of desoxycorticosterone acetate to pregnandiol in man.[7]

6. Gaunt, R., and Hays, H. W.: Science **88**:576, 1938. Corey, E. L.: Am. J. Physiol. **132**:446, 1941. Emery, F. E., and Greco, P. A.: Endocrinology **27**: 473, 1940. Pfeiffer, C. A., and Hooker, C. W.: Am. J. Physiol. **131**:441, 1940. Collings, W. D.: Endocrinology **28**:75, 1941.

7. Cuyler, W. K.; Ashley, C., and Hamblen, E. C.: Endocrinology **27**: 177, 1940.

In addition to the alphaketol structure of the two carbon side chain, the alpha, beta unsaturated ketone on carbon 3, $\Delta^{4,5}$ has been shown to be essential for physiologic activity.[8]

The steroid derivative which has the glyoxal grouping on C_{20}-C_{21} has some activity,[9] but the corresponding glycol [10] and the hydroxyaldehyde derivatives [11] have very little physiologic activity (fig. 2). The conversion of the primary alcohol group on carbon 21 to an ester modifies the activity, depending on the organic acid used. The ester of corticosterone made with diethylacetic acid is the most active of those investigated. The butyric acid ester is the most active of those made from the straight chain acids.[12]

Corticosterone contains four atoms of oxygen and has an atom of hydrogen on C_{17}. Closely related to this structure is a series of compounds which contain five atoms of oxygen and have a hydroxyl group in place of the hydrogen on C_{17}. Compound S (Reichstein's series) is C_{17}-hydroxydesoxycorticosterone [13] and compound E (author's series) is C_{17}-hydroxy-C_{11}-dehydrocorticosterone.[5b]

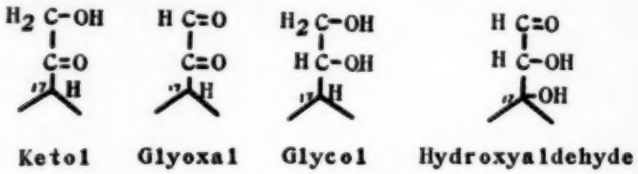

Figure 2.

The isolation of some of the active substances of the adrenal cortex in crystalline form raised a problem which had not been exhaustively investigated in other closely related steroid derivatives. One of the atoms of oxygen in both series of compounds, those that contain four and those that contain five atoms of oxygen, is inert. The function of the oxygen and its position remained obscure for some time, but it

8. Shoppee, C. W.: Helvet. chim. acta **23**:740, 1940. Reichstein, T., and Fuchs, H. G.: ibid. **23**:676, 1940. Kendall, E. C.; Mason, H. L.; Hoehn, W. M., and McKenzie, B. F.: J. Biol. Chem. **119**:lvi, 1937. Wettstein, A., and Hunziker, F.: Helvet. chim. acta **23**:764, 1940. Mason and others.[5a]

9. Reich, H., and Reichstein, T.: Helvet. chim. acta **22**:1124, 1939.

10. Reichstein, T., and von Euw, J.: Helvet. chim. acta **22**:1222, 1939.

11. von Euw, J., and Reichstein, T.: Helvet. chim. acta **23**:1114, 1940.

12. Kuizenga, M. H., and Cartland, G. F.: Endocrinology **27**:647, 1940.

13. Reichstein, T.: Helvet. chim. acta **21**:1490, 1938. Reichstein, T.; Meystre, C., and von Euw, J.: ibid. **22**:1107, 1939. Reichstein, T. and von Euw, J.: ibid. **23**:1258, 1940.

was finally shown that the acid derived from corticosterone by treatment with periodic acid responded to oxidation by the formation of a second ketone group.[14] This could occur only if the inert atom of oxygen in corticosterone was present as a secondary alcohol. The only two probable positions for such an alcohol group and the corresponding ketone are 11 and 12.[15] Position 12 was excluded by the preparation of 3,12-diketoetiocholenic acid.[16] This leaves 11 as the most probable position of the inert atom of oxygen in corticosterone and related compounds.

One of the difficulties associated with this phase of the investigation was the fact that steroid derivatives with an atom of oxygen attached to carbon 11 have not been prepared from natural sources except in the case of sarmentogenin. The preparation of this compound has not been repeated since the identity of the seed from which it was separated is now in question. It was originally suggested that digoxigenin possessed an atom of oxygen at position 11, but this has been disproved. The oxygen is attached to carbon 12.[17]

The evidence that a group of active substances of the adrenal cortex possessed an atom of oxygen attached to carbon 11 immediately stimulated speculation in regard to this structure. Reichstein[18] suggested that the steroid derivatives present in the adrenal cortex were prepared from dextrose and that the compounds with an atom of oxygen on carbon 11 represented an intermediate step in the elaboration of the complete hormone. Such a point of view removed any significance attached to an atom of oxygen on carbon 11, but further work has shown that the presence of the additional atom of oxygen in this position is of the greatest importance. The influence of the hormones of the adrenal cortex on carbohydrate metabolism is sharply limited to those compounds which have this structure.

INFLUENCE OF THE HORMONES OF THE ADRENAL CORTEX ON CARBOHYDRATE METABOLISM

The influence of the adrenal cortex on carbohydrate metabolism can be stated briefly, although to establish the results a large amount of experimental work has been required. Adrenalectomy produces

14. Kendall, E. C.; Mason, H. L.; Hoehn, W. M., and McKenzie, B. F.: Proc. Staff Meet., Mayo Clin. **12**:136, 1937.

15. Kendall, E. C.; Mason, H. L.; Hoehn, W. M., and McKenzie, B. F.: Tr. A. Am. Physicians **52**:123, 1937. Steiger, M., and Reichstein, T.: Helvet. chim. acta **20**:817, 1937. Reichstein, T.: ibid. **20**:953, 1937.

16. Mason, H. L., and Hoehn, W. M.: J. Am. Chem. Soc. **60**:2566, 1938.

17. Mason, H. L., and Hoehn, W. M.: J. Am. Chem. Soc. **61**:1614, 1939.

18. Reichstein, T.: Helvet. chim. acta **20**:978. 1937.

instability in carbohydrate metabolism. Various aspects of this can be summarized as follows:

The glycogen content of the liver of a fasting rat is depressed almost to zero after adrenalectomy.[19] Although the fasting adrenalectomized animal cannot retain glycogen in the liver, the hormones of the adrenal cortex are not essential for the deposition of glycogen. A point of view contrary to this statement was expressed by Britton and Corey[20] in a current article, but Long and his associates[21] and Anderson, Herring and Joseph[22] and other investigators[23] have shown that the ability to deposit glycogen in the liver is not impaired in the adrenalectomized rat, provided food is ingested and the proper balance of inorganic ions is maintained. There is a difference in the response of different animal species because of differences in appetite and habits, but these are of quantitative, not qualitative, nature.[21]

The administration of an unfractionated extract of the adrenal cortex to an adrenalectomized animal causes prompt deposition of glycogen in the liver, even in fasting animals.[24] Perfusion of a cat's liver with a solution containing dextrose and gum acacia does not bring about deposition of glycogen unless an extract of the adrenal cortex is added to the perfusate.[25] In the presence of the extract the concentration of glycogen in the liver is rapidly increased as much as 100 per cent.

The concentration of dextrose in the blood of fasting adrenalectomized animals is low but is restored to normal on ingestion of food or by administration of an extract of the adrenal cortex.[26]

Not only does the extract of the adrenal cortex conserve carbohydrate through deposition of glycogen in the liver, but it also retards the combustion of dextrose and there is a depression in the respiratory quotient.[26]

After Partial Pancreatectomy or Hypophysectomy.—An extract of the adrenal cortex increases the percentage of available carbohydrate which is excreted by partially depancreatized rats if they ingest food. Glyconeogenesis is also stimulated, and the ratio of the increased amount

19. Britton, S. W., and Silvette, H., in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, p. 357.

20. Britton, S. W., and Corey, E. L.: Am. J. Physiol. **131**:790, 1941.

21. Long, C. N. H.; Katzin, B., and Fry, E. G.: Endocrinology **26**:309, 1940.

22. Anderson, E.; Herring, V., and Joseph, M.: Proc. Soc. Exper. Biol. & Med. **45**:488, 1940.

23. Wells, B. B., and Kendall, E. C.: Unpublished data.

24. Sprague, R. G.: Proc. Staff Meet., Mayo Clin. **15**:291, 1940.

25. (*a*) Corey, E. L., and Britton, S. W.: Am. J. Physiol. **131**:783, 1941. (*b*) Britton and Corey.[20] (*c*) Long, Katzin and Fry.[21] (*d*) Wells and Kendall.[23]

26. Thorn, G. W.; Koepf, G. F.; Lewis, R. A., and Olsen, E. F.: J. Clin. Investigation **19**:813, 1940. Long, Katzin and Fry.[21]

of carbohydrate to the increased amount of nitrogen excreted in the urine indicates that the source of the dextrose is protein.[21]

In hypophysectomized fasting rats an extract of the adrenal cortex increases the concentration of the dextrose in the blood above normal, and glycogen is deposited in the liver and to a lesser extent in the muscles. The administration of an extract of the adrenal cortex to hypophysectomized, partially depancreatized rats increases the concentration of the blood sugar and the excretion of dextrose and nitrogen. Glycogen is deposited in the liver but not in the muscles.[21]

The administration of an extract of the anterior lobe of the pituitary gland to hypophysectomized, partially depancreatized rats is followed by a decrease in the concentration of dextrose in the blood and a decrease in the excretion of nitrogen. In partially depancreatized, adrenalectomized rats an extract of the anterior lobe of the pituitary gland does not cause an exacerbation of the diabetes, but the simultaneous administration of extracts of both the anterior lobe of the pituitary gland and the adrenal cortex produces a marked elevation of the concentration of dextrose in the blood and an exacerbation of the diabetes.[21]

The striking effects on carbohydrate metabolism which have been obtained with the whole extract of the adrenal cortex are produced by corticosterone and related compounds, but more significant than this is the fact that the influence on carbohydrate metabolism is limited specifically to the group of compounds which have an atom of oxygen on carbon 11. The amorphous fraction, progesterone, desoxycorticosterone [27] and compound S (Reichstein's series), which is 17-hydroxydesoxycorticosterone, do not affect the deposition of glycogen in the liver and have but slight effect on glyconeogenesis.

After Treatment with Phlorhizin.—The influence of the hormones of the adrenal cortex on glyconeogenesis has also been demonstrated in rats made diabetic with phlorhizin.[28] These results are even more striking than those obtained on partially depancreatized rats, since the rats were maintained in a fasting condition and all dextrose which was excreted in the urine was of endogenous origin.

Phlorhizin administered to an adrenalectomized rat which is fed is well tolerated. The available carbohydrate in the diet is rapidly converted to dextrose and excreted, but if the conversion of protein to sugar is limited to the endogenous protein a great strain is placed on the adrenalectomized rat. Phlorhizin is poorly tolerated, the excretion of dextrose is reduced to about 25 per cent of the amount excreted by a

27. Ingle, D. J., and Thorn, G. W.: Am. J. Physiol. **132**:670, 1941. Long, Katzin and Fry.[21] Thorn and others.[26]

28. (*a*) Evans, G.: Am. J. Physiol. **114**:297, 1936. (*b*) Wells, B. B.: Proc. Staff Meet., Mayo Clin. **15**:294, 1940. (*c*) Long, Katzin and Fry.[21]

normal phlorhizinized rat, and after thirty-six to forty-eight hours convulsions are frequently observed. Death follows within a short interval.[29]

If corticosterone or related compounds are given to a fasting adrenalectomized rat treated with phlorhizin, the amount of dextrose produced from endogenous protein is equal to that produced in the normal fasting animal after treatment with phlorhizin. There is a great loss in body weight, and the volume of the urine is much increased, but in spite of the rapid breakdown of endogenous proteins and the excretion of dextrose the condition of the animal is strikingly improved by administration of corticosterone and related compounds. The animal survives for a much longer interval and at the end of forty-eight or seventy-two hours is brought back to normal on ingestion of food.[29a]

In rats with phlorhizin diabetes, as in partially depancreatized rats, there is a marked qualitative difference among the active substances of the adrenal cortex. Compound E produces a maximal effect which is closely approximated by corticosterone and 11-dehydrocorticosterone. Desoxycorticosterone produces an increase in the amount of dextrose and nitrogen excreted in the urine, but the rats treated with this substance do not survive longer than the untreated animals, and they frequently have convulsions. The amorphous fraction has little effect and sodium chloride is without effect.[29]

Further investigation has shown that the thyroid gland is an important factor which modifies the rate of glyconeogenesis in phlorhizinized-adrenalectomized rats.[30] In phlorhizinized thyroidectomized-adrenalectomized rats the amount of dextrose excreted in the urine after the administration of corticosterone or related compounds is limited by the amount of thyroxin made available, and in the presence of adequate amounts of thyroxin the glycosuria is limited by the amount of corticosterone or related compounds which is available. The administration of adequate amounts of corticosterone or related compounds and thyroxin raises the excretion of dextrose up to or above the normal value.

In hypophysectomized rats it would be anticipated that the absence of the thyrotropic hormone would limit the amount of dextrose excreted after the administration of corticosterone or related compounds. This was found to be true. The amount of dextrose excreted was not restored to normal by the administration of corticosterone unless thyrotropic hormone was also given.[31]

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29. (*a*) Wells, B. B., and Kendall, E. C.: Proc. Staff Meet., Mayo Clin. **15**: 565, 1940. (*b*) Wells.[28b]

30. Wells, B. B., and Kendall, E. C.: Proc. Staff Meet., Mayo Clin. **15**:493, 1940.

31. Wells, B. B., and Chapman, A.: Proc. Staff Meet., Mayo Clin. **15**:503, 1940.

In a recent article [32] the low ratio of dextrose to nitrogen found in adrenalectomized rats after treatment with phlorhizin was assumed to indicate the combustion of dextrose, but work of Wells in my laboratory shows that a ratio of dextrose to nitrogen of 3.3 to 3.6 can be obtained in adrenalectomized rats after treatment with phlorhizin provided the rats are maintained in good condition.[29a] Even under the best experimental conditions a large percentage of the rats die before forty-eight hours unless treated with cortical compounds.

Thyroxin increased the ratio of dextrose to nitrogen to 4.0 or above and hypophysectomy decreased the ratio to 2.2. The full significance of these observations cannot be given at this time.[29a]

Associated with a diabetic state after partial pancreatectomy or administration of phlorhizin there is a ketosis more or less marked, dependent on the amount of fat in the diet. Ingle [33] observed the production of severe ketosis after the administration of compound E to partially depancreatized rats. These rats, however, were maintained on a generous diet which contained 20 per cent of fat. After the administration of phlorhizin to adrenalectomized fasting rats, ketosis is increased by the administration of corticosterone and related compounds, but their condition is improved.

If fat in the form of olive oil is given to fasting adrenalectomized rats treated with phlorhizin, there is but little increase in the degree of ketosis unless corticosterone or related compounds are administered. Under these conditions large amounts of desoxycorticosterone acetate also produce an increase in the amount of keto acids excreted.[34] This is in contrast to the effect of desoxycorticosterone acetate on glyconeogenesis. The administration of large amounts of desoxycorticosterone acetate decreases rather than increases the glycosuria in fasting adrenalectomized-phlorhizinized rats.[29a]

An extract of the adrenal cortex has an antagonistic effect on insulin. The administration of the whole extract of the adrenal cortex will counteract the hypoglycemic state and convulsions produced by insulin.[35] Further investigation has shown that this action is limited to corticosterone and related compounds. Desoxycorticosterone and the amorphous fraction do not exert an anti-insulin effect. The relation between the active principles of the adrenal cortex and insulin is discussed further on page 500.

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32. Lewis, R. A.; Kuhlman, D.; Delbue, C.; Koepf, G. F., and Thorn, G. W.: Endocrinology **27**:971, 1940.

33. Ingle, D. J.: Proc. Soc. Exper. Biol. & Med. **44**:176, 1940.

34. Wells, B. B., and Kendall, E. C.: Proc. Staff Meet., Mayo Clin. **16**: 113, 1941.

35. Grattan, J. F., and Jensen, H.: J. Biol. Chem. **135**:511. 1940.

The concentration of glycogen in the liver of the normal rat is increased if the partial pressure of oxygen is reduced. This was shown by Evans,[36] but his initial observations have not been followed by further investigation. Evans also showed that in adrenalectomized animals a reduction in the partial pressure of oxygen did not cause an increase of glycogen in the liver, but he did not restore the ability to deposit glycogen by the administration of extracts of the adrenal cortex then available. In view of the results obtained on the deposition of glycogen with corticosterone and related compounds, there seems little doubt that the administration of these compounds to an adrenalectomized rat restores the ability to deposit glycogen in a low partial pressure of oxygen.

CAPACITY OF MUSCLE TO RESPOND TO STIMULATION

A close parallelism has been shown to exist between the influence on carbohydrate metabolism and the capacity of muscle to respond to stimulation. The capacity of muscle to perform work has been investigated principally by Ingle, although other recent work has been carried out by Winter and Knowlton.[37] Ingle found that corticosterone, dehydrocorticosterone and compound E all increase the ability of muscle to respond to long-continued stimulation. These results become even more significant after the demonstration that the amorphous fraction, desoxycorticosterone, and all compounds which do not have an atom of oxygen on carbon 11 have but little effect on muscle. At the present time it is not possible to give a detailed interpretation of the findings, but there is an unbroken parallelism between the two effects: The hormones of the adrenal cortex which affect the deposition of glycogen and glyconeogenesis also markedly increase the capacity of muscle to respond to stimulation. Those hormones which do not affect carbohydrate metabolism have little effect on the capacity of muscle for work.

PHYSIOLOGIC EFFECT OF DESOXYCORTICOSTERONE AND THE AMORPHOUS FRACTION

Although corticosterone and related compounds affect the distribution and excretion of electrolytes, this is true only when they are administered in large amounts. The compounds which influence primarily the distribution and excretion of electrolytes are not the same as those that affect primarily the carbohydrate metabolism.[38] In the extract of the adrenal cortex the distribution of the various active principles is such

36. Evans, G.: Am. J. Physiol. **110**:273, 1934.

37. (*a*) Ingle, D. J.: Endocrinology **26**:478 and (*b*) 472, 1940; (*c*) **27**: 297, 1940; (*d*) Am. J. Physiol. **129**:278, 1940. (*e*) Ingle, D. J., and Kendall, E. C.: Proc. Soc. Exper. Biol. & Med. **45**:602, 1940. (*f*) Winter, C. A., and Knowlton, G. C.: Am. J. Physiol. **131**:465, 1940.

38. Kendall, E. C.: J. A. M. A. **116**:2394, 1941.

that more than 90 per cent of the physiologic activity which is concerned with the maintenance of life and the distribution of electrolytes and water is produced by the amorphous fraction. It is therefore necessary to examine the physiologic activity of these principles somewhat more in detail.

The administration of enormous amounts of the amorphous fraction which has been freed from corticosterone and related compounds does not produce a toxic effect and does not modify significantly the normal concentration of inorganic ions in the plasma. In contrast to this action, desoxycorticosterone acetate produces a decrease in the concentration of potassium in the plasma and an increase in the concentration of the sodium and chloride ions.[39] Associated with these changes, the rat and dog may show prostration with extreme weakness of the muscles,[40] and Selye has shown that if large doses are given intraperitoneally, anesthesia may be produced in the rat.[41] These acute effects last for only a few hours and are not followed by other important changes, but in those patients with Addison's disease who have impaired cardiac function severe symptoms leading even to death may be the result of the retention of sodium and chloride ions and the decrease in the potassium ion.[42] Edema and hypertension have been produced in patients with Addison's disease by the administration of large amounts of desoxycorticosterone acetate.[43] The administration of sodium chloride aggravates the condition, but the effect can be controlled by the administration of small daily doses of desoxycorticosterone acetate and a diet moderately high in potassium and low in sodium content.

The influence of desoxycorticosterone acetate on potassium has been utilized by Truszkowski and Duszyńska[44] to demonstrate a protection against the toxic effects of the potassium ion in normal and adrenalectomized mice.

The influence of desoxycorticosterone acetate on the distribution of sodium, chloride and potassium is due in large part to the acetyl ester of the primary alcohol group on carbon 21. Desoxycorticosterone in the free form has much less effect than does the ester.[39]

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39. Wells, B. B., and Kendall, E. C.: Proc. Staff Meet., Mayo Clin. **15**: 133, 1940.

40. Kuhlmann, D.; Ragan, C.; Ferrebee, J. W.; Atchley, D. W., and Loeb, R. F.: Science **90**:496, 1939. Ragan, C.; Ferrebee, J. W.; Phyfe, P.; Atchley, D. W., and Loeb, R. F.: Am. J. Physiol. **131**:73, 1940.

41. Selye, H.: Proc. Soc. Exper. Biol. & Med. **46**:116, 1941.

42. Wilder, R. M.: Proc. Staff Meet., Mayo Clin. **15**:273, 1940. Tooke, T. B.; Power, M. H., and Kepler, E. J.: ibid. **15**:365, 1940. Loeb, R. F.: Bull. New York Acad. Med. **16**:347, 1940. Thorn, G. W., and Firor, W. M.: J. A. M. A. **114**:2517, 1940. Gordon, E. S.: ibid. **114**:2549, 1940.

43. Soffer, L. J.; Engle, F. L., and Oppenheimer, B. S.: J. A. M. A. **115**:1860, 1940.

44. Truszkowski, R., and Duszyńska, J.: Endocrinology **27**:117, 1940

The important position in the animal organism which is held by the amorphous fraction of the adrenal cortex is shown by the effect of this fraction on renal function and on the maintenance of life in adrenalectomized animals. Although this fraction does not have a significant effect on carbohydrate metabolism, it contains by far the most active principle in the adrenal cortex when the criterion for physiologic activity is renal function. The kidney expresses the type of reaction which is modified by the amorphous fraction and although the influence of this fraction is probably exerted through the entire animal organism, a quantitative study of its effect on the transfer of ions is most clearly demonstrated in the kidney.

The amount of the amorphous fraction required to maintain normal renal function in an adrenalectomized dog is between 1 and 2 micrograms per kilogram of body weight per day. This is between fifty and a hundred times less than the amount of corticosterone and about five hundred times less than the amount of compound E required to maintain a normal concentration of urea, sodium and potassium in the blood.

QUALITATIVE DIFFERENCES IN THE PHYSIOLOGIC EFFECTS OF THE HORMONES OF THE ADRENAL CORTEX

Growth.—The important influence of the adrenal cortex on growth was shown by Hartman and Thorn [45] a short time after an active extract of the adrenal cortex had been prepared. Subsequently Ingle [46] found that the administration of large amounts of an extract of the adrenal cortex prevented a gain in weight by older rats and Wells and Kendall [39] clearly demonstrated striking qualitative differences in the effects of the various active substances of the adrenal cortex. The influence of the amorphous fraction is directly opposite to that of corticosterone and related compounds.

The administration of the amorphous fraction to young normal or adrenalectomized rats will increase their rate of growth. The daily administration of 1 mg. of compound E to a similar group of rats will retard somatic growth. The retardation applies not only to the body weight but also to the long bones.[47] Watson and Williams [48] have made a study of the phosphatase content of the epiphyses of the bones and have shown that the amorphous fraction has little effect on the phosphatase content but that corticosterone and compound E produce a decrease in the phosphatase content.[48b]

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45. Hartman, F. A., and Thorn, G. W.: Proc. Soc. Exper. Biol. & Med. **28**: 94, 1930.

46. Ingle, D. J.: Endocrinology **24**:194, 1939.

47. Wells, B. B., and Kendall, E. C.: Proc. Staff Meet., Mayo Clin. **15**:324, 1940.

48. (*a*) Watson, E. M.: Endocrinology **27**:521, 1940. (*b*) Williams, H. L., and Watson, E. M.: ibid. **29**:250, 1941.

Associated with the changes in somatic growth there is atrophy of the adrenal and thymus glands in normal rats.[49] Corticosterone and related compounds which have an atom of oxygen on carbon 11 produce marked atrophy of the adrenal glands of male rats, and, if given in sufficiently large doses, in the adrenal glands of female rats.[46] In addition, this series of compounds will produce almost complete atrophy of the thymus gland. In the interpretation of these results, modification in the structure of the active substances of adrenal cortex must be kept in mind. Progesterone,[50] testosterone and estrone will cause atrophy of the thymus gland, and it is possible that the active substances of the adrenal cortex when given in large amounts are changed at least in part so that sufficient amounts of the sex hormones are produced to bring about an effect on the thymus gland.

In contrast to corticosterone and related compounds, the amorphous fraction even in large amounts does not produce significant atrophy of either the adrenal or the thymus gland.[39]

Resistance.—Qualitative differences among the active compounds in the adrenal cortex have been shown in the effects of these compounds on the resistance of rats and dogs to trauma and to the influence of toxic substances. Selye and associates have found that corticosterone provides the greatest protection against the toxic effect of formaldehyde and traumatic shock; desoxycorticosterone is not effective.[51] Since changes in blood volume and in concentration of inorganic ions are associated with shock, there is little doubt that the active substances of the adrenal cortex will provide a form of therapy that will be of great value at least in some types of shock.[52] Swingle and associates [53] recently have shown that desoxycorticosterone acetate will successfully protect the adrenalectomized dog against circulatory collapse and shock after the intraperitoneal injection of an isotonic solution of dextrose and also against the toxic effects of large amounts of epinephrine and trauma to the muscle. However, when the intestines were stripped, desoxycorticosterone acetate did not prevent the development of shock but contributed

49. (*a*) Ingle, D. J.; Higgins, G. M., and Kendall, E. C.: Anat. Rec. **71**: 363, 1938. (*b*) Ingle, D. J.: Proc. Soc. Exper. Biol. & Med. **44**:174, 1940. Wells and Kendall. [39]

50. Clausen, H. J.: Endocrinology **27**:989, 1940.

51. Selye, H.; Dosne, C.; Bassett, L., and Whittaker, J.: Canad. M. A. J. **43**:1, 1940.

52. Selye, H., and Dosne, C.: Lancet **2**:70, 1940. Fine, J.; Fuchs, F., and Mark, J.: Proc. Soc. Exper. Biol. & Med. **43**:514, 1940. Ragan, C.; Ferrebee, J. W., and Fish, G. W.: ibid. **42**:712, 1939. Perla, D., and Marmorston, J.: Endocrinology **27**:367, 1940; Natural Resistance and Clinical Medicine, Boston, Little, Brown & Company, 1940.

53. Swingle, W. W.; Hays, H. W.; Remington, J. W.; Collings, W. D., and Parkins, W. M.: Am. J. Physiol. **132**:249, 1941.

to the prostration and weakness of the muscles. Corticosterone, on the other hand, did prevent shock when this was produced by stripping of the intestine.

The toxic effects of histamine and the activity of histaminase in adrenal deficiency have been investigated by Browne and associates[54] and Wilson.[55] Administration of the whole extract of the adrenal cortex restores the histaminase activity in adrenalectomized rats, but the effects of the different active compounds in the adrenal cortex have not yet been investigated.

The resistance of the animal organism to toxic substances typified by typhoid vaccine has been investigated by Ettelson.[56] Administration of the whole extract of the adrenal cortex confers a high degree of resistance on adrenalectomized rats and protects them against injected typhoid vaccine. Desoxycorticosterone is not effective, but compound E (author's series) has recently been shown to be very active. Not more than 0.03 mg. of compound E is required to protect an adrenalectomized rat against 25 minimal lethal doses of typhoid vaccine. The influence of corticosterone and of the amorphous fraction is under investigation.

Adrenalectomized rats are sensitive to a low environmental temperature. Confinement for a few hours at a temperature near 0 C. will cause death,[57] but the administration of as little as 13 micrograms of the amorphous fraction will permit survival. The amorphous fraction, however, is not unique in this respect; approximately 16 micrograms of corticosterone and 18 to 20 micrograms of compound E also protect adrenalectomized rats against a low temperature.[38]

RELATION BETWEEN STRUCTURE AND PHYSIOLOGIC EFFECT

The substances which affect carbohydrate metabolism can be segregated from those that influence renal function. They are characterized by an atom of oxygen attached to carbon 11. This modification of the molecule does not endow the active compound with added properties but drastically modifies the physiologic effect of the whole molecule. The action on renal function is suppressed; the effect on the liver is enormously increased.

It is now possible to compare the effect of a hydroxyl group on carbon 17 with that of one on carbon 11. Desoxycorticosterone does not have a hydroxyl group on either carbon 11 or carbon 17. Compound S

54. (*a*) Karady, S.; Rose, B., and Browne, J. S. L.: Am. J. Physiol. **130**:539, 1940. (*b*) Rose, B., and Browne, J. S. L.: ibid. **131**:589, 1941.

55. Wilson, A.: J. Physiol. **99**:241, 1941.

56. Ettelson, L. N.: Endocrinology **27**:340, 1940.

57. Selye, H., and Schenker, V.: Proc. Soc. Exper. Biol. & Med. **39**:518, 1938.

(Reichstein's [13] series) has the structure of desoxycorticosterone plus a hydroxyl group on carbon 17. This compound affects renal function slightly less than does desoxycorticosterone and has no effect on carbohydrate metabolism or on the capacity of muscle for work. Corticosterone has a hydroxyl group on carbon 11 but none on carbon 17. Corticosterone affects carbohydrate metabolism and muscular activity but has little action on renal function.

METHODS OF ASSAY OF THE ACTIVE SUBSTANCES OF THE ADRENAL CORTEX

The large number of physiologic effects produced by the compounds extracted from the adrenal cortex and in particular the sharp separation of the qualitative effects of the various compounds emphasize the necessity of using suitable criteria for assay of these substances. Assay based on a single type of response is not adequate. Several methods have been suggested; twelve will be reviewed briefly.

1. Survival.—The criterion first suggested and used for the assay of cortical principles was the survival of adrenalectomized rats, cats, dogs and guinea pigs. This is useful to demonstrate the presence of compounds with cortical activity, but it is not specific for the determination of the various components.[58] Both the amorphous fraction and many of the crystalline compounds which affect primarily carbohydrate metabolism will also maintain the life of adrenalectomized animals. In the use of this criterion it is essential to control the intake of sodium, chloride and potassium ions. Ninety per cent of a group of adrenalectomized rats may die if the group is given a diet high in potassium and low in sodium, but 90 per cent of a second group of the same stock of rats may survive adrenalectomy if the group is given a diet high in sodium and low in potassium.[37d]

2. Growth of Young Rats.[45]—For highly purified preparations of the amorphous fraction, the influence on the rate of growth of young rats is significant, but the directly antagonistic effect of corticosterone and 17-hydroxy-11-dehydrocorticosterone and their acetates, which may cause not only a retardation in growth but an actual loss in weight, indicates that it is impossible to use growth of young rats as a general criterion for the assay of extracts of the adrenal cortex.[59]

3. Survival of Adrenalectomized Rats in a Low Environmental Temperature.[57]—This is probably the most sensitive criterion for the determination of the presence of compounds which have cortical activity.

58. Kuizenga, M. H.; Nelson, J. W., and Cartland, G. F.: Am. J. Physiol. **130**:298, 1940. Schachter, R. J., and Bebee, M. O., Jr.: Proc. Soc. Exper. Biol. & Med. **40**:541, 1939. Clark, W. G.: ibid. **46**:253, 1941.

59. Ingle and Kendall.[37e] Wells and Kendall.[39]

It is, however, nonspecific: Both the amorphous fraction and corticosterone and related compounds are active in exceedingly small concentrations. It is also probable that the test may be affected by factors other than the active substances of the adrenal cortex.

4. Maintenance of a Normal Condition in Adrenalectomized Dogs.—This method of assay has been proved to be the most reliable for the determination of the activity of all of the extracts of the adrenal cortex. Although both the amorphous fraction and the crystalline compounds will maintain a normal condition in adrenalectomized dogs, the results are not misleading and they become specific for the compound used when considered with respect to the quantity required. When the highly purified amorphous fraction is tested, as little as 1 to 2 micrograms per kilogram of body weight is sufficient. Although the results obtained with corticosterone or related compounds are equally satisfactory, the minimal amounts of these compounds which are required are so enormously greater that the results are not misleading.

This method was devised by Pfiffner, Swingle and Vars.[60] At that time it was concluded that the amount of any given solution required per kilogram of body weight by a series of adrenalectomized dogs did not vary more than 100 per cent. Further investigation of this aspect of the method in my laboratory has shown this conclusion to be incorrect. In a large series of dogs the variations have been as great as thirty times, and variations of from 300 to 600 per cent are frequently encountered. However, if a series of dogs is standardized as closely as possible with desoxycorticosterone, an extract of the adrenal cortex or the highly purified amorphous fraction and the basal requirement of each dog is determined, this series of animals forms a very satisfactory colony for the subsequent standardization of the active substances of the adrenal cortex in relation to renal function.

5. Influence on the Toxic Action of Potassium Salts.—The specific action of desoxycorticosterone acetate on the toxic effects of potassium has been used by Truszkowski and Duszyńska [44] to assay desoxycorticosterone acetate. It is doubtful if the observation can be used with other substances but as regards this synthetically prepared compound the percentage of the animals which survive the injection of potassium chloride and the logarithm of the dose of desoxycorticosterone acetate bear a straight line relationship when amounts of less than 0.5 mg. of desoxycorticosterone acetate are used.

6. Changes in the Concentration of Sodium and Potassium in the Serum.—The only compound which significantly modifies the concentration of sodium, chloride and potassium in the plasma is desoxycorticos-

60. Pfiffner, J. J.; Swingle, W. W., and Vars, H. M.: J. Biol. Chem. **104:** 701, 1934.

terone acetate.[61] This method does not have wide application, but it is necessary to include this response to distinguish the effect of desoxycorticosterone acetate from that of the amorphous fraction.

7. Deposition of Glycogen in Fasting Adrenalectomized Rats.—The glycogen in the liver of a fasting adrenalectomized rat is rapidly depleted, and after eighteen to twenty-four hours the concentration is close to 0.2 per cent. The administration of corticosterone and related compounds will cause deposition of glycogen, which continues for many hours even in the fasting animal,[62] and although this method has not been suggested, it appears to hold great promise for the assay of this group of compounds. Desoxycorticosterone and other substances of the adrenal cortex which do not possess an atom of oxygen on carbon 11 do not increase the concentration of glycogen.

8. Glyconeogenesis After Administration of Phlorhizin in Adrenalectomized Rats.—A second method which can be used to show the influence on carbohydrate metabolism is to measure the amount of dextrose excreted by the adrenalectomized rat after treatment with phlorhizin. This method has not been suggested for the assay of this group of compounds, but the results are specific. The compounds which do not affect carbohydrate metabolism are sharply differentiated.[29a]

9. Anti-Insulin Effect.—The anti-insulin effect of the compounds which influence carbohydrate metabolism is highly specific. Although small differences may not be detected, this effect would serve as a suitable qualitative test to show the presence of corticosterone and related compounds. The method as originally devised by Grattan and Jensen [35] is to inject small amounts of insulin intraperitoneally into mice which have been previously treated with the extracts of the adrenal cortex.

10. Short Stimulation of Muscle.—In much of the work carried out in European laboratories the Everse-De Fremery test [63] has been used. This consists in the maintenance of adrenalectomized rats for some days on the solution to be assayed. The response of the muscle to stimulation is then determined over a short interval of time. The normal muscle will respond characteristically both as to intensity and as to duration. The muscle of the adrenalectomized animals gives an equally characteristic response, which is distinguished by the inability of the muscle to respond in respect to both intensity and duration. For reasons which are not at all apparent, this method can be used with certain compounds but not with others. Desoxycorticosterone acetate produces a maximal effect. On the other hand, compound E, which is the most active in the

61. Wells and Kendall.[39]

62. Long, Katzin and Fry.[21] Wells and Kendall.[23]

63. Everse, J. W. R., and De Fremery, P.: Acta brev. neerland. **2**:152, 1932.

Ingle test, produces a feeble response in the Everse-De Fremery test.[64] This method is so highly selective that it cannot be recommended for the assay of preparations of the adrenal cortex.

11. Long Stimulation of Muscle.—Ingle [65] has devised a method which is in sharp contrast to that of Everse and De Fremery. Rats are adrenalectomized and are placed immediately on a board suitable for the support of the muscle which is to be stimulated three times a second with a silver electrode embedded directly in the muscle. The rat is anesthetized with phenobarbital, and the stimulation is continued until the muscle becomes exhausted. This will occur within a few hours unless the animal is treated with a suitable extract from the adrenal cortex. This test distinguishes the amorphous fraction and desoxycorticosterone, both of which have little effect, from the crystalline compounds which have an atom of oxygen on carbon 11. Only those compounds which affect carbohydrate metabolism maintain a high capacity for work in the muscle, and this method appears to be specific for this group of compounds.[37e]

12. Swimming Test.—A second method of assay which is based on the capacity of muscle for work is furnished by the swimming test.[66] A weight is tied to the tail of an adrenalectomized rat that has been maintained with an extract or a crystalline compound from the adrenal cortex. The animal is then placed in a tank of warm water and allowed to swim until exhausted. The time required is noted. There are differences in the time required for rats that have been maintained with the various active substances of the adrenal cortex. The method has not been extensively used, however, and the results have not been related to the qualitative effects of these substances.

FUNCTIONS OF THE ADRENAL CORTEX

Although a large number of physiologic processes are modified by absence or overabundance of the hormones of the adrenal cortex, an important problem which is still under investigation is concerned with the primary effects of these hormones. The results of investigations have been difficult to interpret because until quite recently the qualitatively different physiologic effects of the hormones of the adrenal cortex have not been recognized, and attempts to formulate the function of the adrenal cortex by a single theory which included the influence on the distribution and excretion of inorganic ions as well as that on carbohydrate metabolism have not been successful. However, if the

64. Reichstein, T.: Helvet. chim. acta **19**:1107, 1936.

65. Ingle, D. J.: Am. J. Physiol. **116**:622, 1936.

66. Gaarenstroom, J. H.; Waterman, L., and Laqueur, E.: Acta brev. neerland. **7**:10, 1936.

influence on the distribution and excretion of inorganic ions is limited to certain hormones, and the influence on carbohydrate metabolism is also limited to quite distinctly different hormones, the principal difficulties are removed. It immediately becomes evident that the adrenal cortex has more than one function.

The known physiologic processes which are affected by the hormones of the adrenal cortex can be grouped in three divisions. These are:

1. The control of permeability and the transfer of inorganic ions and water between extracellular and intracellular phases and from blood to urine.

2. The activity of tissues with specialized functions, such as liver, kidney, gastrointestinal tract and muscle. After adrenalectomy these physiologic processes are disturbed in large part because of changes in permeability and in the distribution of water and inorganic ions, especially sodium and potassium.

3. The activation of enzymes.

Before the functions of the adrenal cortex can be considered in these three divisions, it is necessary to discuss briefly the condition of adrenal deficiency. Removal of the adrenal cortex is followed by a series of events which have been reviewed in the introduction of this paper. The most significant feature of the changes is their progressive nature. Under the best experimental conditions, in which the intake of inorganic salts is properly controlled, there may be a change from normal which is so gradual that a functional test is required to demonstrate adrenal deficiency.

If the intake of inorganic ions is not optimal, the departure from the normal state may be so rapid that an acute deficiency is reached in a few hours, and the severity of the symptoms rapidly increases until crisis and death. The question to be answered is, What is the primary change?

1. Desoxycorticosterone and the amorphous fraction of the adrenal cortex have the greatest effect on the distribution and excretion of inorganic ions. These compounds furnish a highly efficient mechanism which is concerned with the transfer of inorganic ions between tissues and fluids and from the blood to the urine.

During the early stages of deficiency the primary effect so far as the kidney is concerned appears to be on the tubules[67]; water and inorganic ions pass through the glomeruli without impairment. In the tubules, however, the absence of the hormones prevents the reabsorption of the sodium and chloride ions and at the same time permits increased absorption of the potassium ion. As the adrenal deficiency progresses there is finally an effect on the glomeruli, and eventually anuria occurs.

67. Harrison, H. E., and Darrow, D. C.: Am. J. Physiol. **125**:631, 1939.

The progressive nature of these changes presents the most important reason why the results of investigations have been differently interpreted. After the secondary irreversible changes have impaired renal function, it is no longer possible to determine the details of the primary effects of adrenal deficiency. But by the use of extracts or of the crystalline substances of the adrenal cortex adrenalectomized animals can be maintained in excellent condition and the influence of renal function can be studied. Such investigations have shown that the excretion or the retention of inorganic cations and anions is modified by the various hormones of the adrenal cortex, and they indicate a specific effect which is related to the structure of each hormone. Furthermore, the influence of an excess of any of the various hormones on the threshold for the excretion of inorganic salts is specific and is related to the structure of the hormone.

The full significance of these results cannot be interpreted at this time, but it is evident that the effects of the various hormones cannot be explained by restoration of the metabolic processes to normal. There is evidence that the hormones are closely concerned with permeability, and changes which are characteristic for each hormone can be shown without the development of symptoms of adrenal deficiency. As an example of this may be mentioned the administration of compound E (author's series) to an adrenalectomized dog maintained with daily doses of desoxycorticosterone acetate or the change from adequate doses of desoxycorticosterone acetate to adequate amounts of corticosterone, of the whole extract or of the amorphous fraction. All of these experiments clearly indicate the primary and direct influence exerted on permeability and the transfer of inorganic ions from blood to urine by the hormones of the adrenal cortex.[68]

Another example of the influence which the hormones of the adrenal cortex exert on permeability and the transfer of inorganic ions is the rate at which sodium ions enter fluid injected into the peritoneal cavity of the normal animal. Cantarow and Rakoff have shown that the intraperitoneal injection of a 5.5 per cent solution of dextrose is followed by the entrance of sodium into this solution at a rate which is increased 100 per cent during the first five minutes by desoxycorticosterone acetate. After thirty minutes the concentration of sodium in the peritoneal fluid is the same with or without the presence of desoxycorticosterone acetate.[69]

Other evidence for the influence on capillary walls is furnished by the work of Swingle.[53] He concluded that the hemoconcentration

68. Wells, B. B.; Bollman, J. L.; Mann, F. C., and Kendall, E. C.: Unpublished data.

69. Cantarow, A., and Rakoff, A. E.: Endocrinology **27**:652, 1940. Remington, J. W.: ibid. **26**:631, 1940.

observed during adrenal deficiency is due to the absence of the adrenal hormones which are essential for the integrity of the capillary bed. In the absence of the hormones the normal volume of the circulating blood cannot be maintained. The addition of an extract of the adrenal cortex alone, without extra water or sodium chloride, restored the normal state of the capillary bed.

That the permeability of the capillaries is modified by an extract of the adrenal cortex has also been shown by Menkin.[70] An inflammatory exudate produces an increase in the permeability of the capillaries, which is manifested by the accumulation from the circulation of a blue dye in a cutaneous region in which the exudate previously has been injected. This effect of the exudate on the permeability of the capillaries is inhibited wholly or in part by an extract of the adrenal cortex which may be added to the exudate when it is injected or may be injected separately several minutes or hours previously.

2. The narrow limits within which the concentration of inorganic ions is maintained in the blood and tissues indicate the importance of these mineral constituents. The essential character of the influence of inorganic ions on biochemical reactions must be probed further before the relationships can be appreciated, but many examples of the importance of this influence are available. Fenn [71] has recently reviewed the role of potassium in physiologic processes. The injection of subtoxic amounts of potassium salts into normal rats raises the concentration of blood sugar, decreases the concentration of glycogen in muscle and liver and retards the deposition of glycogen during an injection of dextrose.[72]

When cat's liver is perfused with a solution of dextrose which contains gum acacia and an extract of the adrenal cortex, glycogen is deposited, but if 1 per cent of potassium acetate is added to the perfusate, glycogen is not deposited in the liver.[25a] McQuarrie and co-workers [73] have found that the concentration of dextrose in the blood and the severity of glycosuria in a case of diabetes are modified by the ingestion of salts of sodium and potassium.

It has recently been shown that potassium causes a 40 to 50 per cent rise in the respiration of muscle tissues.[74]

The work of Miller and Darrow [75] indicates that the concentration of potassium ions in the muscle cannot be related to the capacity to respond to tetanic stimulation, but there is a close relation between the

70. Menkin, V.: Am. J. Physiol. **129**:691, 1940.
71. Fenn, W. O.: Physiol. Rev. **20**:377, 1940.
72. Silvette, H.; Britton, S. W., and Kline, R.: Am. J. Physiol. **122**:524, 1938.
73. McQuarrie, I.; Thompson, W. J., and Anderson, J. A.: J. Nutrition **11**:77, 1936.
74. Kleinzeller, A.: Biochem. J. **34**:1241, 1940.
75. Miller, H. C., and Darrow, D. C.: Am. J. Physiol. **129**:264, 1940.

liberation of potassium from the muscle and the influence of the hormones of the adrenal cortex.

Fenn and co-workers have shown that potassium is liberated during the stimulation of muscle. These observations have been extended by the observations that when acetylcholine is injected potassium is liberated from the muscle.[76] If muscle is denervated, the potassium which is released is increased tenfold, but the curarized muscle does not react to acetylcholine and does not liberate potassium. After adrenalectomy the contraction of muscle and the liberation of potassium by acetylcholine appear to depend on the condition of the animal and the degree of adrenal deficiency. These results and the effect of corticosterone and related compounds on the response of muscle indicate the relationship of both potassium and certain of the hormones of the adrenal cortex to muscular activity.[77]

The importance of potassium in biochemical reactions is shown by the effect of dextrose on the distribution of this ion in the animal organism and in a suspension of yeast in water. The intravenous injection of dextrose will cause a decrease in the concentration of potassium in the serum of a dog, and if dextrose is added to a suspension of yeast, potassium passes from the solution into the cells until fermentation of the dextrose occurs, at which time the potassium again passes into the solution.[78]

The progressive nature of the condition of adrenal deficiency ultimately involves the rate of many chemical reactions. The rate of glyconeogenesis is rapidly reduced about 75 per cent, and the formation and utilization of keto acids likewise decrease.[79] In vitro the consumption of oxygen in kidney from adrenalectomized rats is much below that of normal kidney tissue, and the rate of deaminization may be suppressed as much as 25 per cent in kidney tissue taken from adrenalectomized animals.[80] Absorption and transfer of inorganic ions through the intestinal tract are not normal,[81] and the disturbances in water and

76. Cicardo, V. H., and Moglia, J. A.: Nature, London **145**:551, 1940; Rev. Soc. argent. de biol. **16**:54, 149 and 554, 1940. Verzár, F., and Somogyi, J. C.: Nature, London **145**:781, 1940.

77. Hoff, H. E.; Winkler, A. W., and Smith, P. K.: Am. J. Physiol. **131**: 615, 1941. Miller, H. C., and Darrow, D. C.: ibid. **132**:801, 1941. Stickney, J. C.: ibid. **132**:9, 1941.

78. Flock, E.; Bollman, J. L.; Mann, F. C., and Kendall, E. C.: J. Biol. Chem. **125**:57, 1938. Pulver, R., and Verzár, F.: Helvet. chim. acta **23**:1087, 1940.

79. Nelson, N.; Grayman, I., and Mirsky, I. A.: J. Biol. Chem. **132**:711, 1940. Wells and Kendall.[29a]

80. (*a*) Crismon, J. M., and Field, J., II: Am. J. Physiol. **130**:231, 1940. (*b*) Tipton, S. R.: ibid. **132**:74, 1941. (*c*) Russell, J. A., and Wilhelmi, A. E.: J. Biol. Chem. **137**:713, 1941.

81. (*a*) Stein, L., and Wertheimer, E.: Proc. Soc. Exper. Biol. & Med. **46**: 172, 1941. (*b*) Dennis, C., and Wood, E. H.: Am J. Physiol. **129**:182, 1940.

electrolytes which are found in the blood and tissues of adrenalectomized animals are so intimately related with metabolic activity that it has been difficult to determine which is primary and which secondary.[82]

The administration of the active substances of the adrenal cortex will restore to normal all of these disturbed processes but, even more significant, the control of the intake of sodium, chloride and potassium will maintain a normal concentration of the inorganic constituents of the blood; absorption through the intestinal tract is adequate for maintenance, deaminization is sufficiently rapid to permit the utilization of protein, renal function is restored to normal when measured by the concentration of urea in the blood, and blood pressure and volume of the blood are within normal limits.[83]

All of these results which are obtained either by control of the intake of sodium, potassium and chloride ions or by administration of the active substances of the adrenal cortex emphasize the importance of inorganic ions to the activity of enzymes.

3. The administration of corticosterone and related compounds to normal or fasting animals is followed by deposition of glycogen in the liver.[84] This effect appears to involve activation of enzymes, and experiments in vitro have shown that it takes place within an interval of fifteen minutes.[25a]

The deposition of glycogen in the liver is the best example of the activation of enzymes by cortical hormones. Whether there is a direct action between the hormone and the enzyme system or whether the effect is mediated through inorganic ions has not been made certain, but it has been shown that potassium ion has an important if not dominating effect.

The experiments which show the influence of the hormones of the adrenal cortex on other enzymes are less direct, and they have all been in vivo. Russell and Wilhelmi [80c] have confirmed the earlier work of Jiménez-Díaz and have shown that the kidney tissue of an adrenalectomized rat cannot produce ammonia as rapidly as the normal kidney. The addition of desoxycorticosterone acetate in vitro is without benefit, but the administration of desoxycorticosterone acetate to an adrenalectomized rat will restore the kidney tissue to normal in this respect.

82. Muntwyler, E.; Mellors, R. C., and Mautz, F. R.: J. Biol. Chem. **134**: 345, 1940. Muntwyler, E.; Mellors, R. C.; Mautz, F. R., and Mangun, G. H.: ibid. **134**:367, 1940. Harrison, H. E., and Darrow, D. C.: J. Clin. Investigation **17**:77, 1938.

83. Anderson, E.; Joseph, M., and Herring, V.: Proc. Soc. Exper. Biol. & Med. **44**:477 and 482, 1940. Anderson, E., and Joseph, M.: ibid. **46**:321, 1941. Cleghorn, R. A.; Armstrong, C. W. J., and Austen, D. C.: Endocrinology **25**: 888, 1939. Kendall.[4c]

84. Britton and Corey.[20] Long, Katzin and Fry.[21]

Histaminase is present in lower concentration in adrenalectomized rats, but its normal activity is restored by the whole extract of the adrenal cortex.[85] Examples of enzyme action influenced by corticosterone and related compounds are the conversion of protein to dextrose and the formation of ketone acids in the liver. In adrenalectomized rats the rates of these reactions are restored to normal by the hormones of the adrenal cortex.[86]

EVIDENCE OF THE INFLUENCE OF THE HORMONES OF THE ADRENAL CORTEX ON A SPECIFIC CHEMICAL REACTION

As the investigation of the functions of the adrenal cortex has progressed, it has become evident that no single physiologic process is involved, and it therefore follows that the symptoms of adrenal deficiency cannot be due to failure of any one specific chemical reaction. However, during the early years of the investigation this evidence was not available, and attempts were made to identify the function of the adrenal cortex with control of the rate of a single chemical reaction. Two of these hypotheses will be discussed, not because they have been shown to be correct but because they have usefully provided an avenue of approach for experimental work and because they have been cited and accepted as probable if not final expressions of the function of the adrenal cortex.

In order even to be considered as a possible function of the adrenal cortex, any single chemical reaction would have to be of such a nature that it is essential for cellular activity. Such a reaction is phosphorylation, and this was chosen by Verzár for investigation.[87] His experiments indicated that adrenalectomized rats could be maintained with riboflavin phosphate but not with riboflavin alone. The results were interpreted to show the inability for phosphorylation in an adrenalectomized rat, and this hypothesis was then expanded to explain the changes in the intestinal tract and in carbohydrate metabolism and all aspects of hypofunction in adrenal deficiency.

The experiments of Verzár have been repeated, but the results have not upheld the original hypothesis. Neither riboflavin nor its phosphoric ester will maintain the lives of adrenalectomized rats, and the hormones of the adrenal cortex are not essential for the elaboration of the yellow enzyme or for other reactions which involve phosphorylation.[88]

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85. Karady, Rose and Browne.[54a] Rose and Browne.[54b] Wilson.[55]

86. Wells and Kendall.[29a] Wells and Kendall.[34]

87. Verzár, F.: Die Funktion der Nebennierenrinde, Basel, Benno Schwabe & Company, 1939.

88. Bruce, H. M., and Wien, R.: J. Physiol. **98**:375, 1940. Nelson, D.: Am. J. Physiol. **129**:P429, 1940.

Thiamine is phosphorylated in the adrenalectomized rat, and the cozymase content of the tissues of adrenalectomized rats does not differ from that of normal animals.[89] The failure of the intestine to absorb dextrose at its normal rate may be explained on grounds other than failure to phosphorylate. It is undoubtedly true that the rate of phosphorylation is decreased as the symptoms of deficiency increase, but this decline in rate is not confined primarily to phosphorylation, and there is no sound evidence to support the hypothesis that the hormones of the adrenal cortex are essential for phosphorylation.[90]

The second hypothesis for the function of the adrenal cortex which was based on a specific chemical reaction was suggested by Jiménez-Díaz. He found that the capacity of the kidney to make ammonia was greatly decreased, and at the time this work was carried out this loss of function assumed such importance that it was considered to be the primary change in the development of adrenal deficiency.[91] Jiménez-Díaz suggested that the inability to form ammonia resulted in the loss of sodium and that this in turn produced the changes in distribution and excretion of electrolytes. The specific effects of the various hormones on renal function show that the primary change is not concerned with the formation of ammonia and the many examples of alterations in the distribution of inorganic ions, as in the intestinal tract,[81b] which cannot be secondary to failure to form ammonia remove support from the hypothesis of Jiménez-Díaz.

Recently the effect of the adrenal cortex on another specific chemical reaction has been studied. Thorn and associates [32] have investigated the formation of dextrose from lactic and pyruvic acids and alanine in adrenalectomized rats after treatment with phlorhizin. Because of a decreased rate of formation of dextrose they suggested the hypothesis that the adrenal cortex is concerned with this phase of carbohydrate metabolism. There is no question that conversion of the compounds with three atoms of carbon into dextrose was not at a normal rate in their experiments, but other work has shown that the conditions under which such an experiment is carried out are of the greatest importance, and when they are more nearly optimal, the conversion of exogenous protein to carbohydrate in adrenalectomized rats treated with phlorhizin is at a rate close to normal.[29a]

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89. Ochoa, S., and Rossiter, R. J.: J. Physiol. **97**:1P, 1939-1940. Runnstrom, J.; Sperber, E., and Bárány, E.: Nature, London **145**:106, 1940.

90. Houssay, B. A.; Foglia, V. G., and Fustinoni, O.: Rev. Soc. argent. de biol. **15**:139, 1940. Marazzi, R.: Am. J. Physiol. **131**:36, 1940. Barnes, R. H.; Miller, E. S., and Burr, G. O.: J. Biol. Chem. **140**:241 and 247, 1940.

91. Jiménez-Díaz, C.: Lancet **2**:1135, 1936.

THE RELATION BETWEEN THE HORMONES OF THE ADRENAL CORTEX AND THOSE OF OTHER ENDOCRINE GLANDS

The effects produced by the hormones of the adrenal cortex are dependent on the quantitative relationships of other hormones. With insulin there is antagonism.[92] This has been shown by the greatly increased sensitivity of adrenalectomized rats and dogs to insulin and by the increased protection afforded by corticosterone and related compounds against convulsions produced by insulin. The anti-insulin effect appears to be a specific action between the hormones of the adrenal cortex and insulin. In the fasting adrenalectomized rat, after the administration of phlorhizin the administration of corticosterone and related compounds prevents the development of convulsions which presumably are due to insulin. The anticonvulsive effect is not due to an increase in either the concentration of dextrose in the blood or the content of glycogen in the liver.[29a]

In the normal, fed rat corticosterone and related compounds do not produce glycosuria, but in the absence of insulin these compounds increase the excretion of dextrose and accelerate the rate of glyconeogenesis. The over-all result of the experiment can be simply stated, but the finely controlled balance, which undoubtedly includes the pituitary gland and the pancreas, is only partially revealed. A study of the metabolic process which involves the conversion of protein to carbohydrate shows the highly important but well concealed part played by insulin.[93]

The drop in blood sugar, which in some cases may be the cause of death after adrenalectomy, may be due to the relative excess of insulin after sudden removal of the hormones of the adrenal.

In hypophysectomized rats the administration of corticosterone and related compounds does not affect the glycogen in the muscle unless an extract of the anterior lobe of the pituitary gland is also given. There appears to be a synergism between the hormones of the adrenal cortex and those of the anterior lobe of the pituitary gland; either group alone is without effect.[94]

The depression of the oxidation of dextrose by corticosterone and related compounds is likewise dependent on the secretion of the anterior lobe of the pituitary gland. The administration of corticosterone and related compounds to hypophysectomized rats is without effect on the respiratory quotient unless an extract of the pituitary gland is also injected.[95]

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92. Wells and Kendall.[29a] Grattan and Jensen.[35]

93. Gaebler, O. H., and Galbraith, H. W.: Endocrinology **28**:171, 1941. Wells and Kendall.[29a]

94. Russell, J. A.: Am. J. Physiol. **128**:552, 1940.

95. Greaves, J. D.: Personal communication to the author.

When corticosterone and related compounds are injected, somatic growth is retarded and the adrenal glands of normal rats are atrophied, but administration of an extract containing the adrenotropic hormone of the anterior lobe of the pituitary gland will prevent atrophy of the adrenal glands.[49a]

This and much other evidence shows that the adrenal gland is one of the "target" organs whose activity is modified by the pituitary gland. In a recent review Swann concluded that a function of the pituitary gland may be to preserve the lives and secretory activities of the adrenal cortical cells in the fasciculate and reticular phases of their history.[96] After hypophysectomy, rats do not die from adrenal deficiency, and the distribution and excretion of inorganic ions are not significantly disturbed. However, the changes in carbohydrate metabolism indicate hypofunction of the adrenal cortex. This may be ascribed either to a deficiency in the elaboration of corticosterone and related compounds, which may occur in the innermost layers of the adrenal cortex, or to the lack of the secretion of the pituitary gland which is essential for some of the specific effects of this group of compounds.

96. Swann, H. G.: Physiol. Rev. **20**:493, 1940.

Notes and News

University News, Promotions, Resignations, Appointments, etc.—Eugene L. Opie has become emeritus professor of pathology in the Cornell University Medical College, New York, and John C. Torrey, emeritus professor of epidemiology.

In the University of Oregon Medical School, Warren C. Hunter has been promoted from associate professor to professor of pathology.

Ralph I. Dorfman, research assistant in physiologic chemistry in Yale University, has been appointed assistant professor of biochemistry in Western Reserve University, where he will be associated with the Brush Foundation and the medical department of Lakeside Hospital.

Thomas Francis Jr. has resigned as professor of bacteriology in the New York University College of Medicine to become professor of epidemiology in the recently established school of public health in the University of Michigan.

Colin M. MacLeod, associate of the Rockefeller Institute for Medical Research, has been appointed professor of bacteriology in the New York University College of Medicine.

In Loyola University School of Medicine, Chicago, John F. Sheehan has been advanced to professor of pathology in the place of F. A. McJunkin, retired, and James W. Henry has been appointed assistant professor of pathology and pathologist to Mercy Hospital.

James E. Davis has retired from the professorship of pathology in the Wayne University College of Medicine, Detroit.

Clarence W. Muehlberger, toxicologist to the coroner's office of Cook County, Chicago, has accepted the appointment as director of the newly established crime detection laboratory of the Michigan Department of Health and the Michigan State Police. William D. McNally becomes again toxicologist to the coroner of Cook County, a position which he filled from 1913 to 1929.

Society News.—The Biological Photographic Association, an international group of photographers in the natural sciences, will hold its eleventh annual meeting in the Hotel Buffalo, New York, Sept. 11-13, 1941. For information, write the secretary of the association, Magee Hospital, Pittsburgh.

Obituaries

CHARLES LLOYD CONNOR, M.D.
1891–1941

Charles Lloyd Connor, M.D., professor of pathology at the University of California Medical School, died of hypertensive cardiovascular disease on June 12, 1941.

Born in Robertsdale, Pa., on Oct. 28, 1891, his boyhood was spent in Pennsylvania, where his father, John Snedden Connor, the superintendent of a coal mine, specialized in locating and opening new mines. His early life is the stimulating story of a youth with a goal ahead attaining it under adverse circumstances. Medicine was his goal. A student instructorship in zoology at Valparaiso University assisted him in securing his preliminary education. Work in the steel mills and on the railroad enabled him to enter the University of Pittsburgh, where part time work as a bacteriologist allowed him to continue. In 1918 he married Marjorie Ruthe Forter. She and a daughter, Jean Louis Connor, survive him. After service with the United States Army in Texas, he resumed his medical studies, graduating at Baylor University in 1920. An internship at St. Joseph's Hospital in Pittsburgh was followed by a year of medical practice in Forsythe, Mont. Here, his interest in research was stimulated by intimate contact with the problem of Rocky Mountain spotted fever. A National Research Council fellowship for study of this problem was granted in 1921. This brought him to Harvard University Medical School. Here, under the direction of Prof. S. Burt Wolbach, Dr. Connor began his career as a teacher and investigator. He remained at Harvard as an instructor on the faculty until 1928. During this period, he spent one year as an associate in pathology at McGill University and as director of laboratories at the Montreal General Hospital.

In 1928 he was called to the University of California Medical School, where he served as professor of pathology and chairman of the research committee. A major educational contribution has been his organization of graduate instruction in pathology. Many well qualified pathologists trained by him are serving in various parts of the country, in practice, teaching and research.

Dr. Connor's contributions to medical science include pioneer studies on Rocky Mountain spotted fever, on the nature of normal and abnormal pigments in the body and on malignant tumors, particularly those arising in bone. His most recent research was a fundamental contribution con-

cerning the genesis of cirrhosis of the liver. His conclusion is now widely accepted that the major form, so-called alcoholic cirrhosis of the liver, is related to factors causing long-standing fatty infiltration of the liver, such as diabetes and the presence of poisons that inhibit proper tissue oxidation. Thus, alcohol, particularly when combined with carbohydrate starvation, is an important contributory factor.

CHARLES LLOYD CONNOR, M.D.
1891–1941

The excellent museum of pathology of the University of California Medical School was developed under Dr. Connor's direction. He organized it to be used directly in the teaching not only of pathology but of other branches of medicine. Reflecting particularly Dr. Connor's concern over basic problems of pathology, the museum is especially rich in material dealing with malignant disease and with hepatic, renal and cardiac disorders. He assisted materially in the development of the

systematic study of neoplastic diseases at the University of California Medical School and cooperated in the application of the cyclotron to medical investigation.

The impetus which he has given to education and research at the University of California Medical School will remain as a lasting memorial to his fine unselfish spirit.

Books Received

NATIONAL RESEARCH COUNCIL: REPORT OF COMMITTEE ON DRUG ADDICTION 1929-1941 AND COLLECTED REPRINTS 1930-1941. Cloth. Pp. 1581. Washington, D. C., National Research Council, 1941.

TEXTBOOK OF BACTERIOLOGY. Edwin O. Jordan, Ph.D., late Andrew McLeish Distinguished Service professor of bacteriology, University of Chicago, and William Burrows, Ph.D., assistant professor of bacteriology, University of Chicago. Thirteenth edition, revised. Pp. 731, with 170 figures. Price $6. Philadelphia: W. B. Saunders Company, 1941.

THE COMPLETE WEIGHT REDUCER. C. J. Gerling. Pp. 246. Price $3. New York: Harvest House, 1941.

LYMPHATICS, LYMPH, AND LYMPHOID TISSUE, THEIR PHYSIOLOGICAL AND CLINICAL SIGNIFICANCE. Cecil Kent Drinker, M.D., D.Sc., professor of physiology, Harvard University School of Public Health, and Joseph Mendel Yoffey, M.Sc., M.D., F.R.C.S. (Eng.), senior lecturer in anatomy, University of South Wales and Monmouthshire, Cardiff, Wales. Pp. 406, with 50 figures. Price $4. Cambridge: Harvard University Press, 1941.

PATHOLOGY OF THE ORAL CAVITY. Lester Richard Cahn, D.D.S., associate professor of dentistry (oral pathology) Columbia University, Fellow of the American Association for the Advancement of Science, Fellow of the New York Academy of Dentistry, and associate Fellow of the New York Academy of Medicine. Pp. 240, with 165 figures. Price $5.50. Baltimore: William & Wilkins Company, 1941.

THE ROCKEFELLER FOUNDATION: ANNUAL REPORT, 1940. New York: Pp. 473. The Rockefeller Foundation, 49 West Forty-Ninth Street, 1941.

Book Reviews

Malignant Disease and Its Treatment by Radium. Stanford Cade, F.R.C.S., surgeon, Westminster Hospital, Mount Vernon Hospital and the Radium Institute, London. Fabrikoid. Pp. 1280, with 623 illustrations. Price $18. Baltimore: William Wood & Company, 1940.

The purpose of this book is to further "the more exact and less empirical use of radium" in the treatment of cancer. It is based on the author's own experience and observations. From 1924 to 1939 he had under his care some 4,000 patients with cancer, of whom 3,000 were given radiotherapy alone or in combination with surgical treatment. The work is somewhat one-sided inasmuch as the comparative value of roentgen radiation and radium in combating cancer does not receive much attention. The book will be of special interest to those immediately concerned with the radiotherapy of cancer. The first part, about 100 pages, deals with the physics of radium in chapters as follows: radium; technic of radium therapy (H. F. Flint); radium dosimetry (C. W. Wilson); measurement of radium plaques (L. H. Gray). The second part, 184 pages, discusses radiosensitivity, the mode of action of radiation, the effects of radium on normal and malignant tissues, the dangers of radium and protection against them. The third and main part, about 1,000 pages, describes the malignant tumors of various sites, with special reference to the choice of treatment and the technic of radium treatment. The book is profusely illustrated with photographs of gross and microscopic appearances, as well as with drawings in color. Not all the illustrations are good, but all in all they are highly instructive to the pathologist as well as the radiotherapeutist and clinician. One notes that the tumors of the ovary as now understood do not receive adequate consideration. Also that "carcinoma" and "epithelioma" in many places are used as synonyms. In the interest of a logical and simple usage, "epithelioma" should have been omitted. The pathologist will find a great deal of interest in this book, especially in parts 2 and 3, and it should be available in every tumor clinic.

Biology of the Laboratory Mouse. By the staff of the Roscoe B. Jackson Memorial Laboratory: Clarence C. Little, director; George D. Snell, editor; J. J. Bittner, A. M. Cloudman, E. Fekete, W. E. Heston, W. L. Russell, G. W. Woolley. With a chapter on infectious diseases of mice by J. H. Dingle, Harvard Medical School. Pp. 497, with 172 figures. Price $7. Philadelphia: The Blakiston Company, 1941.

In the preface one is told that in this country not less than a million mice are raised each year for use by investigators in microbiology, cancer and genetics. The purpose of the book, made possible by a special grant from the John and Mary Markle Foundation, is to assist investigators by gathering together in one place scattered information of a fundamental nature about the mouse. The list of the contents will show the ground covered: the early embryology of the mouse (George D. Snell), reproduction (George D. Snell), the histology of the mouse (Elizabeth Fekete), spontaneous neoplasms in mice (Arthur M. Cloudman), gene and chromosome mutations (George D. Snell), the genetics of spontaneous tumor formation and of tumor transplantation (C. C. Little), endocrine secretion and tumor formation (George W. Woolley), the milk influence in tumor formation (John J. Bittner), inbred and hybrid animals and their value in research (W. Lawson Russell), parasites (Walter E. Heston), infectious diseases of mice (John H. Dingle) and care and recording of mice (John J. Bittner). It is pointed out that "in most cases material omitted is adequately covered in other recent books." There is a comprehensive bibliography at the end of every chapter except the one on care and recording. The one on infectious diseases of mice contains 341 references. It is evident from what has been said that this book will be needed in every laboratory in which mice are used regularly in investigative work.